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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 50, No. 1, January 1993 **Volume 50, nº 1, janvier 1993**

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Bruce P. Dancik
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Dispersal and Recruitment of Zebra Mussel (*Dreissena polymorpha*) in a Nearshore Area in West-central Lake Erie: The Significance of Postmetamorphic Drifting

André Martel

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Martel, A. 1993. Dispersal and recruitment of zebra mussel (*Dreissena polymorpha*) in a nearshore area in west-central Lake Erie: the significance of postmetamorphic drifting. Can. J. Fish. Aquat. Sci. 50: 3–12.

Quantitative evidence that early postmetamorphic stages of zebra mussel (*Dreissena polymorpha*) can disperse in the water column and colonize substrates has been obtained using off-bottom scouring pad collectors and plankton sampling in a nearshore, shallow-water area (2–7 m) near Wheatley, Lake Erie, during August 1991. Collectors were deployed for 24–72 h, thus minimizing growth of recruits during deployments. Size criteria determined through an in situ growth experiment were used to discriminate between individuals arriving at a collector as (1) settling larvae or (2) postmetamorphic stages. During certain periods, 20–80% of individuals settling on collectors were postmetamorphic stages. Plankton samples taken near collectors also confirmed the presence of postmetamorphic stages in the water column. Most drifting juveniles ranged from about 300 μm shell length to 800 μm (some up to 1–2 mm). Although settlement by postmetamorphic stages occurred during various periods, they drifted and settled on collectors in much higher numbers during periods of strong wave action generated during storms. Colonization of natural and man-made substrates by postmetamorphic stages may be significant in exposed or turbulent areas and may impact on population dynamics. Whether postmetamorphic drifting is adaptive and which mechanisms are involved are unknown.

Des résultats quantitatifs obtenus en août 1991 ont démontré que les individus aux premiers stades post-métamorphiques de la moule zébrée (*Dreissena polymorpha*) peuvent se disperser dans la colonne d'eau et coloniser des substrats. Ces résultats proviennent de l'échantillonnage du plancton et de l'utilisation de collecteurs constitués de tampons à récurer, déployés près du fond, dans un secteur d'eau peu profonde (2–7 m), placés à proximité du rivage près de Wheatley (Ontario), sur le lac Érié. Les collecteurs étaient submergés de 24 à 72 h, ne permettant ainsi qu'une croissance limitée des recrues durant cette période. Des critères de taille, déterminés lors d'une expérience in situ sur la croissance, ont servi à établir une distinction entre les individus arrivant à un collecteur soit 1) à l'état larvaire (véligère), soit 2) à l'état post-métamorphique (juvénile). De 20 à 80 % des individus qui se fixaient aux collecteurs étaient des juvéniles lors de certaines périodes. Les échantillons de zooplancton prélevés près des collecteurs ont aussi confirmé la présence de juvéniles dans la colonne d'eau. La plupart des juvéniles à la dérive avaient une coquille mesurant entre 300 et 800 μm (parfois jusqu'à 1–2 mm). Bien que des juvéniles se soient fixés à différentes périodes, ces derniers dérivaient et se fixaient aux collecteurs en bien plus grand nombre durant les périodes de fortes vagues produites lors des tempêtes. La colonisation des substrats par les stades post-métamorphiques peut être importante, particulièrement dans les secteurs exposés ou turbulents. Cette colonisation peut aussi avoir des répercussions sur la dynamique des populations de la moule zébrée. On ignore cependant si cette dispersion en phase post-métamorphique est adaptative, ainsi que les mécanismes qui sont en cause.

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Within only 2 yr after its discovery in Lake St. Clair in 1988 (Hebert et al. 1989, 1991) the zebra mussel (*Dreissena polymorpha* (Pallas)) had rapidly colonized vast regions of the Great Lakes, especially downstream, including all of Lake Erie and the western basin and southern shoreline of Lake Ontario (Griffiths et al. 1991). The rapid spread of this small European bivalve is largely explained by the presence of a planktonic larval stage (Griffiths et al. 1991; Mackie 1991; Neary and Leach 1992), which contributes to dispersal of benthic species with complex life cycles (Strathmann 1974; Scheltema 1989).

The presence of a free-swimming planktonic veliger stage in zebra mussels (Lewandowski 1982a; Sprung 1989) contrasts markedly with native freshwater bivalves such as Unionidae, which have specialized short-lived parasitic larvae (glochidia), or Sphaeriidae, which brood small numbers of embryos and have crawl-away juveniles (Mackie 1991). Although the zebra mussel lives in fresh or brackish waters, its life cycle resembles that of many marine bivalves, such as *Mytilus*, which is planktotrophic for several weeks before settling on hard substrates, metamorphosing, and becoming a juvenile. The larval stage is therefore assumed to be the stage during which zebra mussel dispersal and recruitment occur — recruitment referring here to the arrival of the first cohort or age-group that can be accurately censused by an observer in a population survey (see Connell 1985).

Marine molluscs have mechanisms other than the planktonic larval stage by which individuals can disperse in the water column and colonize substrates. One such means is the drifting of early postmetamorphic stages in the water column, as demonstrated in marine bivalves (Bayne 1964; Sigurdsson et al. 1976; Lane et al. 1985) and gastropods (Vahl 1983; Martel and

Chia 1991b) (see also study by Prezant and Chalermwat (1984) on dispersal of the freshwater bivalve *Corbicula fluminea*). The usual mechanism involved in this passive dispersal of postmetamorphic marine bivalves is the production of long microscopic monofilamentlike, drifting mucous threads secreted by the foot (different from the attachment byssus). These mucous threads enable the tiny postmetamorphic stage to drift in the water column with water currents via an increase in hydrodynamic drag (e.g. as in *Mytilus edulis*; see Lane et al. 1985).

Although some authors have addressed the potential significance of postmetamorphic drifting in the life history of *D. polymorpha* as a mechanism of dispersal (Kacanova 1961, cited in Lewandowski 1982b; Griffiths et al. 1991; Mackie 1991), there are, to my knowledge, no published quantitative data on the topic from either the European or North American literature. The aim of this paper is to demonstrate and quantify juvenile drifting in zebra mussels through the use of off-bottom scouring pad collectors deployed for short periods (24–72 h) as well as by plankton sampling in west-central Lake Erie.

Materials and Methods

Description of Study Site

This study was carried out in the west-central basin of Lake Erie, near Wheatley, Ontario, Canada, in the vicinity of the Ontario Ministry of Natural Resources Lake Erie Fisheries Station (42°03′N, 82°27′W) (Fig. 1). Sampling sites surrounded the Wheatley breakwater, situated about 100 m southeast of the entrance to Wheatley Harbour (Fig. 1) where the water is relatively shallow: ~2–3 m deep on the northwest and ~5–7 m deep on the southeast sides, measured ~10 m away from the breakwater. The Wheatley area is protected from predominant westerly winds by Point Pelee (Fig. 1). Easterly winds, in contrast, have a much longer fetch and often result in high wave action, particularly when of long duration from the northeast. Zebra mussels were found on most submerged hard surfaces, including the limestone blocks (~1–3 m^3) of the breakwater.

Deployment of Scouring Pad Collectors

The off-bottom collectors used in this study were made of white-coloured nylon kitchen scouring pads, measuring 12 × 11 cm and 0.6–0.8 cm thick (produced by Fireco/Washrack Co., Mississauga, Ont.). These collectors proved to be very effective in collecting planktonic zebra mussels and yielded high numbers of settlers in short periods when compared with nonfibrous materials commonly used in zebra mussel research (e.g. Plexiglas) (Martel 1992a, 1992b; also see study by King et al. (1990) on the use of scouring pads for studying larval settlement in *Mytilus*). Collectors were kept flat by gluing Plexiglas strips to both the top and bottom part of the scouring pad. A swivel system attached to a hole in the middle of the strips allowed the collector to pivot and exposed the pad's broad surface to the water flow (wave-induced currents) (see Martel 1992a).

New scouring pads were soaked in filtered (64 μm) lake water for 2–3 d before use to remove possible traces of manufacturing chemicals that might have been present on the pad and which could deter initial settlement. Three to five collectors were attached to a vertical polypropylene line in the middle of the water column. The first collector was placed at 50–60 cm above the bottom, with subsequent collectors positioned above the first one at 30- to 50-cm intervals. The line was anchored to the bottom and kept taught by positioning a small buoy just

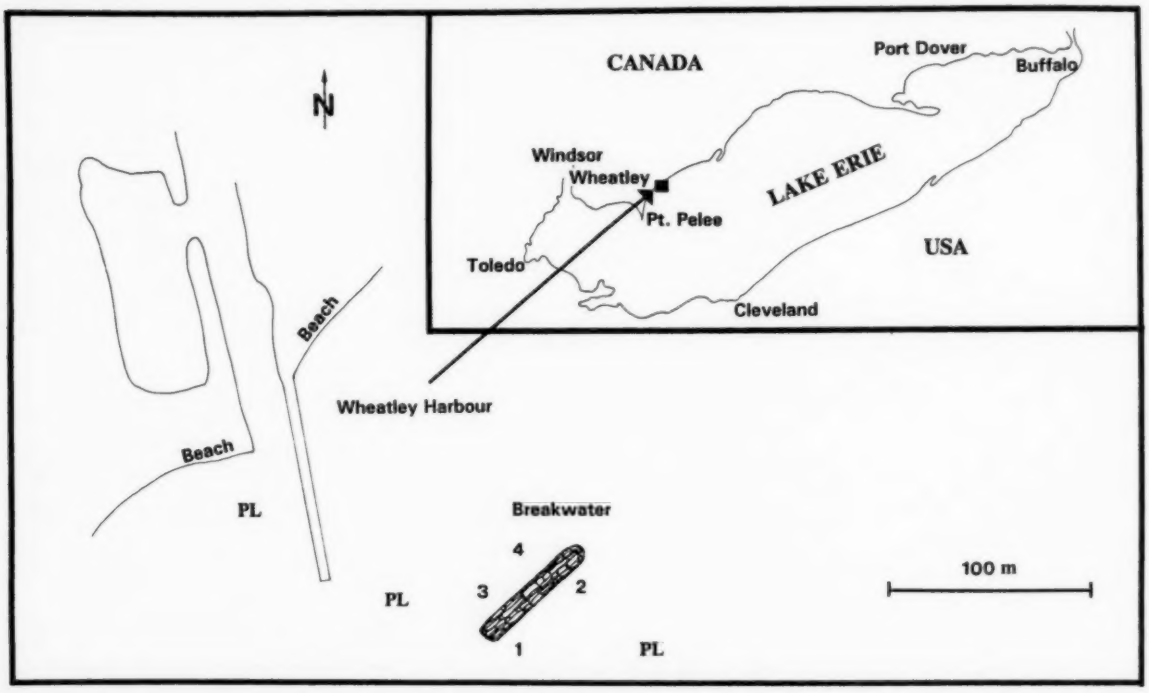

FIG. 1. Sampling sites near the breakwater of Wheatley Harbour, in west-central Lake Erie, Ontario, Canada. Numbers indicate locations where off-bottom scouring pad collectors were deployed and PL the approximate locations where plankton samples were taken.

below the surface which was connected to a larger, surface buoy. For each deployment, two to four such lines were installed at one of four sampling sites around the Wheatley breakwater (Fig. 1). Collectors were deployed during eight periods between August 2 and August 27, 1991, with each deployment lasting from 24 to 72 h: August 2–5 (72 h), August 8–9 (24 h), August 10–11 (24 h), August 14–15 (24 h), August 18–20 (36 and 60 h), August 21–23 (36 h), August 24–25 (24 h), and August 26–27 (24 h). Each deployment was of sufficient duration to allow large numbers of larval as well as postmetamorphic zebra mussels to settle among the nylon fibres of the pad. Upon retrieval, a strong jet of cool tap water was used to extract newly settled larvae as well as small juvenile zebra mussels caught or attached to the fibres of the pad by attachment byssal threads. Both sides of the pad were systematically washed for 15 s each over a large shallow pan and this procedure repeated three times. Individual zebra mussels were then collected by filtration using a set of two sieves with 500- and 200-μm Nitex netting. The filtered zebra mussels were then inspected live under a stereomicroscope before being preserved in 70% ethanol for later counting and measurement using a digitizer connected to a personal computer. For each sample, only those individuals with distinct ciliary bands and gill lamellae were considered to avoid including dead/decaying zebra mussels that occasionally drifted to collectors. When the total number of individuals upon counting and measuring was too large, collector samples were subsampled using a square (6 × 6 cm) dish equipped with a grid (1 × 1 cm). Zebra mussels were measured (umbo-ventral margin measurements) using an Olympus SZH stereomicroscope (at 64×) equipped with a drawing tube and projecting on a Jandel/SigmaScan digitizer system.

Recent studies have shown that at least one other species of zebra mussel is present in the Great Lakes in addition to *D. polymorpha* (May and Marsden 1992). However, adult shells collected in the vicinity of Wheatley during the summer of 1991 fit the description of *D. polymorpha* (A. Martel, unpubl. data). Individuals collected in the present study were predominantly under 1 mm and therefore much too small to enable comparisons of shell shapes, but I assumed that they belong to *D. polymorpha*.

Preliminary in situ Growth Experiment

To establish size criteria that would enable discrimination between zebra mussels that settled on a collector as (1) a larva or (2) a postmetamorphic stage, the growth of newly settled larvae for the periods collectors were deployed (i.e. 24–72 h) was estimated for the first 5 d (120 h) of postmetamorphic life. This growth was determined through an in situ experiment conducted at the same time collectors were deployed. Zebra mussel veligers that had just settled onto a series of scouring pad collectors left overnight (August 21–22, 1991) were used for this experiment, which coincided with a period of high larval settlement in the field. After being left overnight, collectors were brought to the laboratory in plastic bags to prevent newly settled zebra mussels from drying out. Two randomly selected pads were then cut in pieces of approximately 3.5 × 4.5 cm with scissors. Several of these pieces were randomly selected and the newly settled zebra mussels attached to them extracted using pressurized cool tap water. Individuals were then examined and measured under a stereomicroscope to (1) confirm that they had settled on the pads as veligers (overall small size (all <290 μm) and absence of a juvenile portion of the shell) and (2) determine the mean shell length and variance among individuals, which were needed before beginning the growth experiment (called period T_0). The other pieces of nylon pad, which had ~10–40 newly settled zebra mussels attached to the fibres (based on data from other pieces), were individually placed in 5-cm plastic Petri dishes with 64-μm Nitex windows, thus precluding even the smallest zebra mussel veligers from the external environment from entering the dish. A total of 12 such dishes were placed in the field by attaching them to a vertical line in the middle of the water column near the Wheatley breakwater; six dishes were placed on the southeast side and the six others on the northwest side of the breakwater, where water depth was ~2 and 5 m, respectively. Dishes were retrieved 5 d later (period T_{5d}) (one dish was lost). Individual zebra mussels from each dish were then extracted and measured under a stereomicroscope.

Plankton Sampling

Zooplankton samples were taken from both the exposed southeast side and the protected northwest side of the breakwater during the times collectors were deployed. Sampling periods included both calm and stormy (high wave action) weather. Samples were taken using a 200 μm-mesh, 0.58-m-diameter plankton net towed by a small boat. This large-mesh plankton net was used so that only individuals potentially capable of settling on/colonizing substrates were retained, i.e. late-stage veligers (most larvae settle at a body size of over 200 μm (see Lewandowski 1982b)) and postmetamorphic stages. In order to standardize sampling, tows were taken horizontally, for a period of 5 min, moving at ~0.50–0.75 $m \cdot s^{-1}$ (1–1.5 kn) while the net was kept at a constant depth, close to the surface, using a buoy, the top part of the net being 30–50 cm below surface. Zebra mussels collected in the plankton were observed and measured live upon arrival at the laboratory or later after preservation in 70% ethanol.

Results

Preliminary in situ Growth Experiment

All 11 groups of settled zebra mussels retrieved from the separate culture dishes showed significant growth after the 5-d period of the experiment (Mann–Whitney, $p < 0.001$; comparisons between individuals at time T_0 and each of the 11 groups at T_{5d}) (Fig. 2A). Variation between mean shell lengths after 5 d was low for 10 of the 11 groups, thus indicating similar experimental in situ growth conditions. On average, shell length increased from 229 μm (s = 20 μm, n = 263) at time T_0, to 311 μm (s = 34 μm, n = 164) (11 groups pooled) at time T_{5d}, thus an average growth of 82 $\mu m \cdot 5\ d^{-1}$ or 16.4 $\mu m \cdot d^{-1}$ (August 23–28, 1991; 23–25°C) (Fig. 2).

Taking into account a maximal growth of settling larvae for the period scouring pad collectors were deployed, a conservative approach was taken to establish size limit criteria enabling the discrimination betwen zebra mussels that settled on collectors as (1) larvae or (2) drifting juveniles (postmetamorphic stages). This was done by first estimating a maximal possible growth rate for a hypothetical individual, which would have started in the in situ growth experiments (T_0) at a size equivalent to 2 standard deviations (SD) smaller than the mean ((229 μm − 2(20 μm)) = 189 μm) and that would have grown to a size equivalent to 2 SD greater than the mean shell length of individuals after 5 d of growth ((311 μm + 2(34 μm)) = 379 μm). This would represent a theoretical increment of 190 μm in 5 d (379 μm − 189 μm = 190 μm),

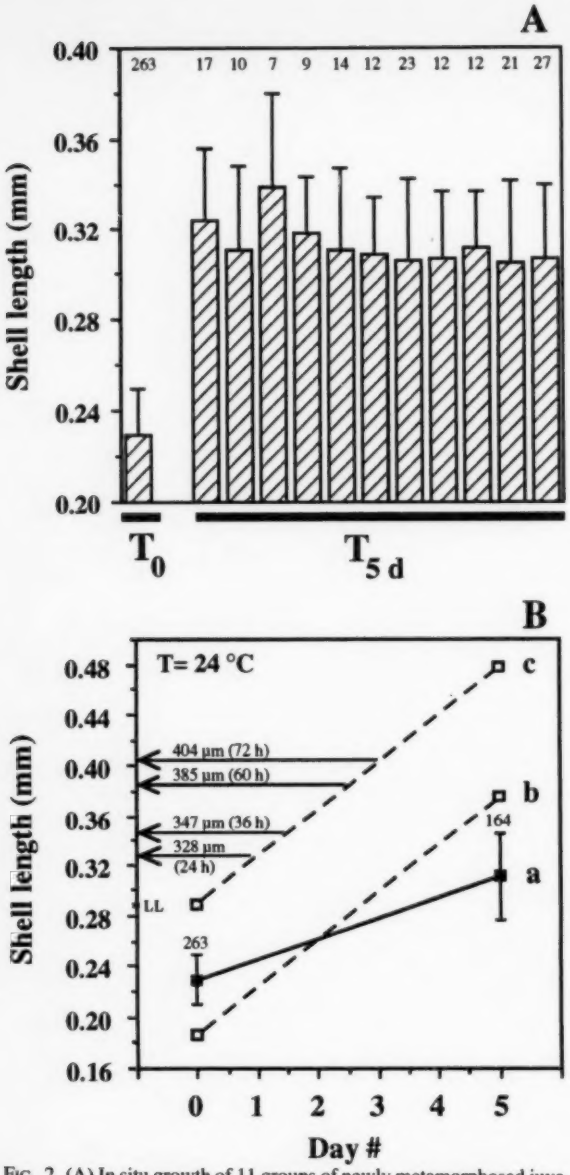

FIG. 2. (A) In situ growth of 11 groups of newly metamorphosed juvenile *D. polymorpha*, August 23–28, 1991; T_0 = mean shell length of all zebra mussels at the beginning of the experiment; T_{5d} = mean shell length of each group after 5 d of growth; vertical error bars represent ± SD. (B) Observed average growth and estimated maximal growth of newly metamorphosed juveniles during the in situ experiment; line *a*: observed average growth of the eleven groups after 5 d; line *b*: estimated maximal growth determined by using mean − 2 SD (at T_0) and mean + 2 SD (at T_{5d}); line *c*: estimated maximal growth determined by using the largest observed settling larva (0.286 mm) and the estimated maximal growth shown with line *b*. Arrows on the left side of the graph indicate the estimated maximal size of settling larvae on collectors after 24, 36, 60, and 72 h deployments.

or 38 µm·d^{-1}, which is 2.3 times greater than the observed mean growth rate (Fig. 2). By giving this maximal daily growth rate (38 µm·d^{-1}) to the largest veliger observed in the plankton during sampling (umbo-ventral margin measurements: 286 µm, rounded at 290 µm), a simple growth line is then drawn and the maximal theoretical sizes of settling larvae growing on the collectors for the time of the deployment can be estimated (Fig. 2B, line *c*). In the present study, the growth of settling larvae for the first days of postmetamorphic life is also assumed to be linear (see study by Bayne (1964) on *Mytilus*). For the periods collectors were deployed, i.e. 24, 36, 60, and 72 h, the maximal theoretical sizes that could be reached by settling larvae were estimated at 328, 347, 385, and 404 µm, respectively (August 23–28, 1991; 23–25°C) (Fig. 2B). To be conservative and simplify calculations, minimal values classifying an individual as a settling postmetamorphic stage or juvenile were set at 330, 350, 390, and 410 µm shell length for deployments of 24, 36, 60, and 72 h, respectively. Therefore, after deployment for a given time period, a zebra mussel removed from the collector and which, upon examination, had a shell length smaller than the calculated minimal juvenile size limit was categorized as having arrived at the collector as a settling larva. An individual with shell length equal to or greater than the size limit could not, in all likelihood, have arrived at the collector as a settling veliger, and therefore must have arrived as a drifting postmetamorphic individual.

Deployment of Scouring Pad Collectors

Analysis of the size frequency distributions (total n = 8014) shows that early postmetamorphic stages of zebra mussels contributed significantly to recruitment to collectors during certain periods (Fig. 3). This is demonstrated by the significant numbers of settling zebra mussels with shell length greater than the maximal size that settling veligers could reach during the time of deploymnent (see vertical broken lines delimitating settling larvae (L) from drifting juveniles (J) shown on each histogram on Fig. 3). In addition to the prodissoconch portion of the shell (prodissoconch = shell of the bivalve veliger), most of these early juvenile zebra mussels showed an unmistakable asymmetrical dissoconch (= juvenile shell portion added to the prodissoconch after metamorphosis of the veliger), clearly indicating that they had already metamorphosed and reached the juvenile stage (postmetamorphic) before arriving at the collectors.

Drifting postmetamorphic zebra mussels were found on the collectors during all eight time periods in which collectors were deployed (Fig. 3, 4). Of the calculated 27 437 zebra mussels that settled on collectors during the eight deployments (95 collectors), as many as 12 657 or 46.13% arrived as drifting postmetamorphic stages. Recruitment rates of postmetamorphic stages were comparable with or greater than those of settling larvae during three of the eight deployment periods: August 8–9, 18–20, and 24–25, with the proportion of recruits arriving as juveniles varying between 30.81 and 78.93% (Fig. 3). These results indicate that during these deployments, postmetamorphic stages drifting in the water column and settling to off-bottom collectors outnumbered settling veligers by a factor of 3:1. These periods of abundant drifting postmetamorphic zebra mussels coincided with periods of high wave action and strong water currents. In the Wheatley area, such periods were usually associated with northeast winds (A. Martel, unpubl. data).

Postmetamorphic zebra mussels were also found on collectors during other deployment periods, including August 2–5, 10–11, 14–15, 21–23, and 26–27, although they composed a much lower fraction of the total number of recruits (Fig. 3). During these periods, the proportion of settling postmetamorphic individuals was low (0–16.51%) and veligers were,

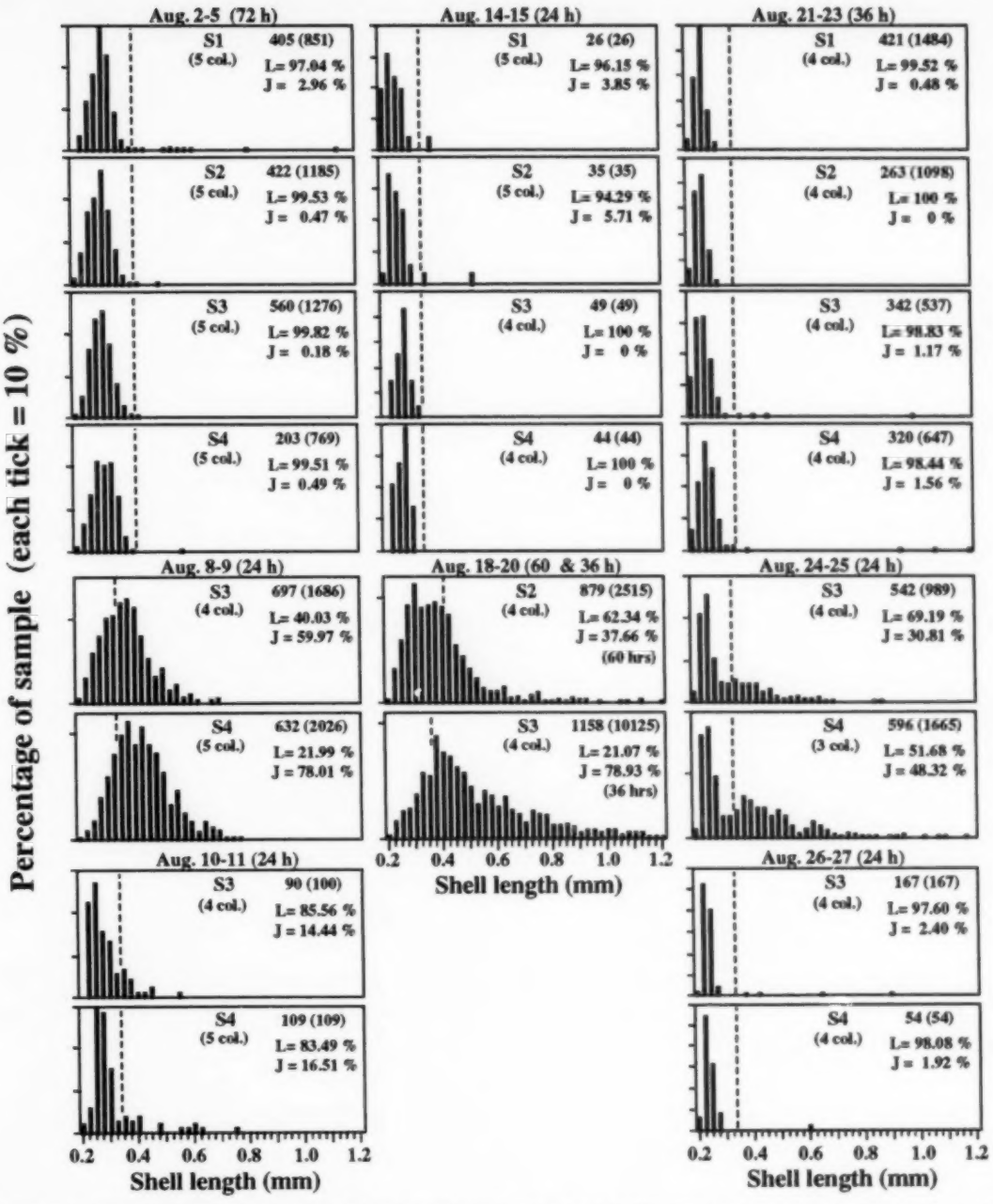

FIG. 3. Size frequency distributions of zebra mussels found in off-bottom scouring pad collectors placed near the Wheatley breakwater (S1–S4) during eight deployment periods in August 1991. Duration of deployment is indicated beside each date (col. = number of collectors deployed). Numbers on the top right inside each histogram represent the number of measured and counted (in parentheses) individuals; L = percentage of recruits arriving as larvae, J = proportion of early juveniles (postmetamorphic); recruits arriving as larvae and as juveniles are also indicated on the left and right side of the vertical broken line, respectively.

by far, the dominant settlers as shown by the high proportions (83–100%) of small individuals and distinct cohorts with shell lengths between 200 and 300 μm. (Fig. 3).

Postmetamorphic zebra mussels collected near the Wheatley breakwater during August 1991 included early juvenile stages with shell lengths predominantly ranging from just over 0.3 to 0.8 mm (Fig. 3, 4). A small but significant proportion of drifting juveniles had shell lengths over 1 mm, with several individuals in the 1.4–1.9 mm range (not included in histograms of Fig. 3).

Drifting early juvenile stages of *D. polymorpha* settled on collectors on 17 of the 22 station days. Their abundance on

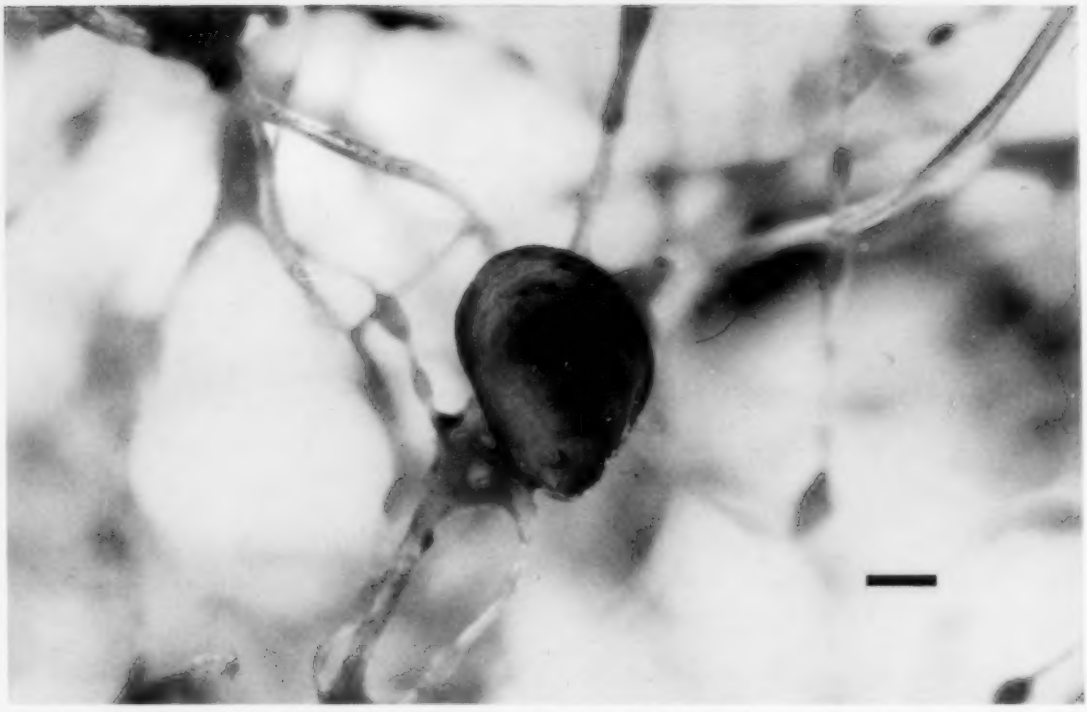

FIG. 4. Photomicrograph of a live postmetamorphic zebra mussel that colonized an off-bottom scouring pad collector deployed for 24 h near the Wheatley breakwater. The zebra mussel is attached to one of the nylon fibres of the scouring pad by its newly secreted attachment byssus (not visible on the picture). The horizontal bar represents 0.2 mm.

collectors and the similarity of size distributions between the different sampling sites for a given deployment period (e.g. S2 and S3 during August 18–20) indicate that dispersal and recruitment of early postmetamorphic zebra mussels occurred on large time and space scales.

Plankton Sampling

Plankton samples taken near the Wheatley breakwater (while collectors were deployed) during August 1991 confirmed the presence of postmetamorphic zebra mussels in the water column (Fig. 5). The occurrence of high numbers of early postmetamorphic zebra mussels in the plankton, and on off-bottom collectors, coincided with periods of high wave action (see plankton samples of August 8 and 19) (Fig. 5, 6). Over 93% of zebra mussels collected in the plankton sample on August 8 with the 200-μm-mesh plankton net were postmetamorphic stages (density of zebra mussels: 7.80 ind.$\cdot m^{-3}$; see Fig. 6). Most individuals were over 300 μm shell length, with the majority being 350–700 μm (only large veligers were retained by the 200-μm-mesh plankton net).

Plankton samples taken on August 19, during stormy weather with strong wave action, at three different locations (Fig. 1) contained drifting juveniles at all three sites (Fig. 6). The percentage of postmetamorphic stages was highest (89.29%) in the sample taken closest to the shore, in the shallow waters of the harbour entrance (har), intermediate (60%) in the sample taken west of the breakwater (bww), and lowest (30.77%) in the sample taken offshore in deeper water on the east side of the breakwater (bwe) (Fig. 6) (mean shell length of individuals: har, 377 μm; bww, 329 μm; bwe, 259 μm; Kruskal–Wallis ANOVA, $p < 0.001$). The bwe sample was taken ahead of the wind-driven water currents and waves during the storm; hence, the presence of drifting juveniles in the water column was not confined to the immediate vicinity of the shore but over a large area.

In contrast, late-stage veligers greatly outnumbered postmetamorphic stages in plankton samples taken during calmer periods (e.g. on August 10 and 22). They represented 92–100% of the total number of individuals collected in the 200-μm-mesh plankton net. During these periods, late-stage veligers were by far the predominant stage at which zebra mussels settled on collectors, as shown by the distinct cohort of small individuals with shell lengths between 200 and 300 μm (Fig. 3). This is reflected by the vast majority of the individuals found in plankton samples being between 200 and 250 μm shell length (Fig. 6). Notice, however, that although collected in small numbers, postmetamorphic zebra mussels were present in plankton samples of August 22, with nearly 8% of the total number of individuals with shell lengths over 300 μm (Fig. 6).

Discussion

Occurrence of Postmetamorphic Drifting in *Dreissena*

The present study reveals that early postmetamorphic stages of the zebra mussel *D. polymorpha* commonly drift in the water column and are capable of colonizing substrates. Although primarily late-stage veligers settled on off-bottom scouring pad collectors during periods of low/moderate wave action, they were outnumbered by drifting early postmetamorphic stages during periods of strong turbulence during storms. Plankton

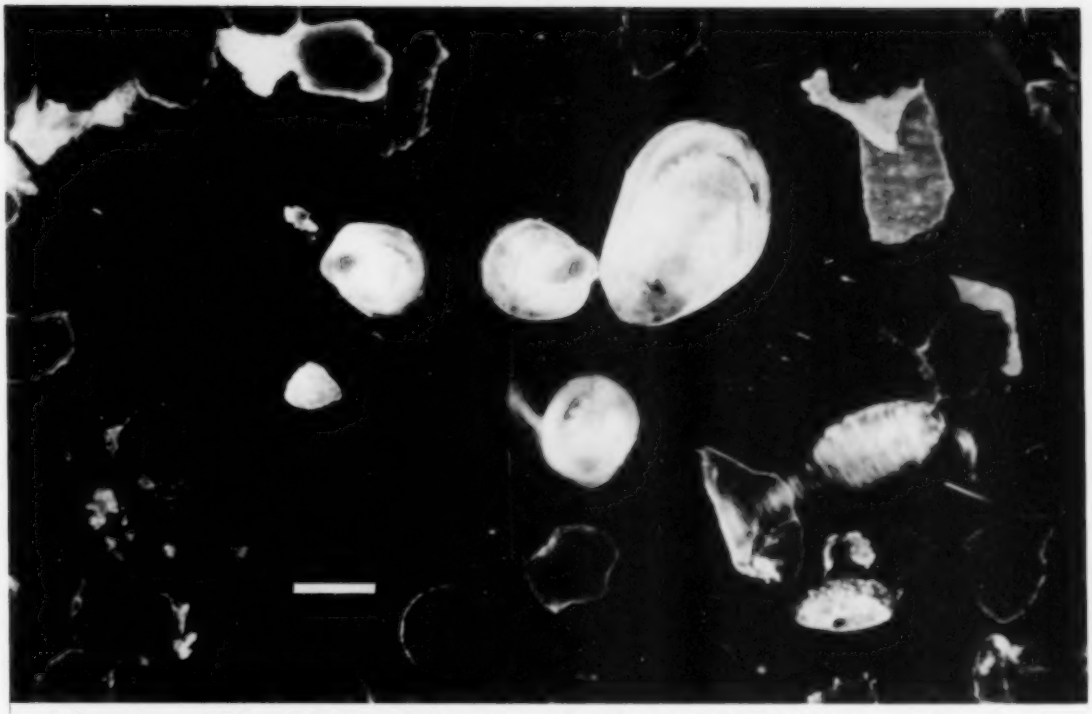

FIG. 5. Photomicrograph of four live postmetamorphic zebra mussels collected in a horizontal plankton sample (200-μm-mesh net) on the west side of the Wheatley breakwater during a northeast storm on August 8, 1991. Notice the typical juvenile shape, especially of the larger individual. The individual at the bottom was protruding its foot (visible on the left side); also compare the size of these early juvenile zebra mussels with that of the copepod. The horizontal bar represents 0.2 mm.

samples taken near collectors confirmed the abundance of drifting postmetamorphic zebra mussels in the water column. Also, the drifting of postmetamorphic stages was not limited to periods of strong wave action or storms, as on several occasions they settled on collectors during periods of relatively calm water, although in much smaller numbers.

Juveniles with shell lengths of 300–900 μm were the most common group in both plankton samples and scouring pad collectors, but many larger juveniles (with shell lengths between 1 and 2 mm) were also present. Based on the data obtained with the in situ growth experiment, drifting juveniles ranged in age from just metamorphosed to 1–2 mo old. Because feeding and growth rates decrease during the fall and winter, it is conceivable that at that time, given suitable hydrodynamic conditions, small-sized zebra mussels (~0.5–2 mm) could be several months old yet still small enough to disperse in the water column. This speculation on the occurrence of older but still small-sized juveniles in the water column is supported by recent collections of postmetamorphic zebra mussels in plankton samples taken during April in Lake Erie (R. Claudi, Ontario Hydro, Toronto, Ont., pers. comm.).

In the present study, a number of factors probably have contributed to the discovery of large numbers of drifting postmetamorphic stages in the water column. Water turbulence due to storms is the first factor, as high numbers of juveniles are likely to be dislodged and resuspended. A second factor is the high catch rate of off-bottom scouring pad collectors, which appear to be highly favourable substrates for settlement and act like passive filters, despite the short period these collectors were deployed. The duration of these deployments was considerably shorter than the 1–2 wk to several months of previous studies on settlement and colonization of zebra mussels (e.g. Lewandowski 1982b; Bij de Vaate 1991; see also Marsden 1992). Such short deployment periods reduce to a minimum the growth of new recruits during deployment and allow the investigator to discriminate between recruits arriving as (1) settling larvae or (2) postmetamorphic stages.

Both the height of the water column and the proximity to dense newly settled colonies may also have had a major influence on numbers as well as sizes of postmetamorphic zebra mussels resuspended and drifting in the plankton. More juveniles were caught in nearshore plankton than offshore on August 19 (Fig. 6). Also, during the same period, collectors deployed closer to the shore and in shallower water (S3 and S4) caught approximately four times more juveniles than the same number of collectors placed in deeper water (S1 and S2) (Fig. 3). Moreover, collectors placed close to the bottom had more postmetamorphic recruits than those nearer the surface (A. Martel, unpubl. data). Thus, studying a nearshore, shallow-water (2–7 m) habitat may have contributed to finding high numbers of drifting postmetamorphic zebra mussels in plankton samples and in off-bottom collectors, especially during storms. Collectors deployed in shallow water may be subject to stronger wave-driven water currents or turbulence, which could result in the resuspension of larger numbers of juveniles that are either weakly attached to hard substrates (e.g. boulders) or attached only to loose substrates (e.g. pieces of macrophytes, adult byssus, detritus; see next section on drifting mechanisms). At

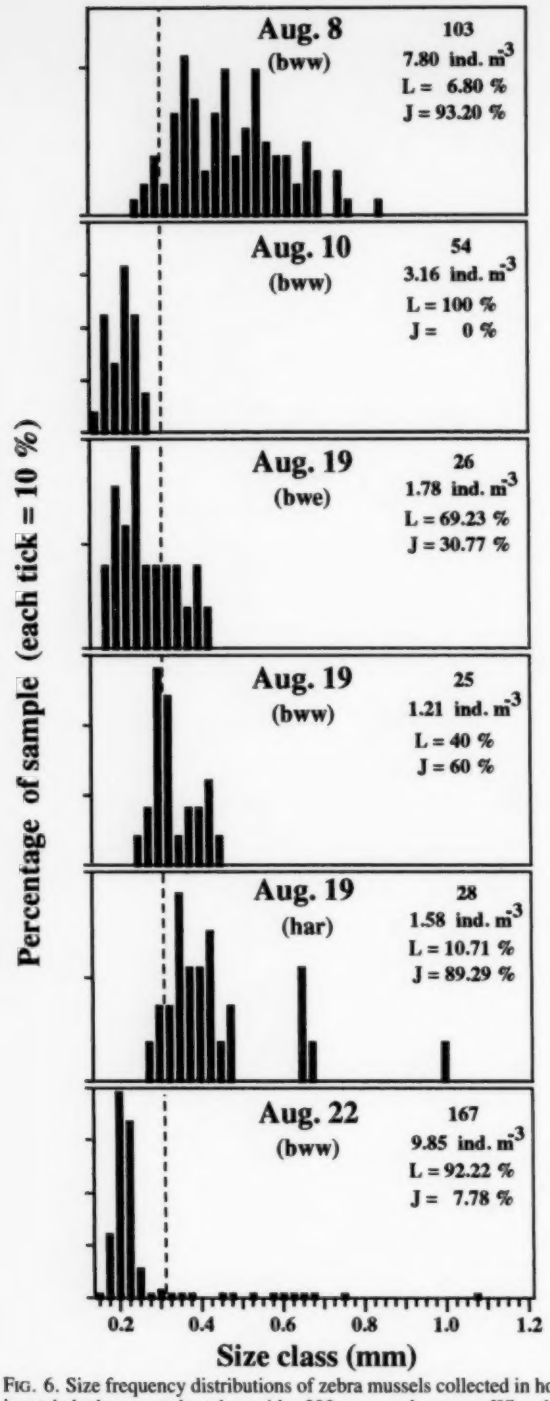

FIG. 6. Size frequency distributions of zebra mussels collected in horizontal plankton samples taken with a 200-μm-mesh net near Wheatley during August 1991. Sample locations: bwe = breakwater east side; bww = breakwater west side; har = mouth of harbour. Numbers on the top right inside each histogram represent the number of individuals measured and their density (ind. m^3 of $water^{-1}$); estimated percentage of individuals found in sample as larvae (L) or drifting early juvenile stages (postmetamorphic) (J) is shown; individuals considered as free-swimming larvae and as drifting juveniles are also indicated on the left and right side of the vertical broken line, respectively.

metamorphosis the veliger larva of the zebra mussel, like that of a marine bivalve with a planktotrophic larval stage, loses its velum. Unlike planktonic larval stages which use the velum to feed and, more importantly, swim to remain in suspension, postmetamorphic zebra mussels placed in a still or low-turbulence water column slowly sink to the bottom. As passive drifters, their presence in the water column is controlled by local hydrodynamic conditions, i.e. water currents, turbulence, and wave action. The higher numbers of postmetamorphic stages found in the plankton as well as in the off-bottom scouring pad collectors during days of high wave action strongly support the view that hydrodynamic conditions play an important role in controlling the density of postmetamorphic stages in the water column.

Although there is no direct evidence, there are reasons to believe that drifting early juvenile stages could disperse over long distances, possibly on the order of hundreds of metres or even several kilometres, particularly if transported by strong wind-driven surface currents. Beukema and de Vlas (1989) reported that postmetamorphic stages of *Macoma balthica*, a small marine bivalve, are caught in offshore plankton samples and can be transported by tidal water currents over 10–15 km away. In the present study, the presence of drifting juvenile zebra mussels in a plankton sample taken on the windward (offshore) side of the Wheatley breakwater during strong easterly winds indicates that juveniles drifting in the water column were not uniquely confined to the immediate vicinity of the breakwater (2–5 m deep) but were also found in deeper (5–7 m) waters away from the shore. It should be noted, however, that so far, postmetamorphic stages drifting in the water column have not been detected in vertical plankton samples taken at offshore stations situated many kilometres off Wheatley (A. Martel, unpubl. data). More intensive offshore sampling during windstorms or periods of high wave action may be necessary to determine whether or not postmetamorphic zebra mussels can drift that far away from the coast and from major zebra mussel colonies. The drifting of postmetamorphic zebra mussels may be largely restricted to coastal, nearshore, or shallow-water habitats (e.g. <10 m), where wave-induced near-bottom water currents are strongest and numbers of small juveniles from nearby colonies greatest. The distance postmetamorphic zebra mussels can disperse remains to be determined and may depend on the mechanism(s) involved during drifting.

Drifting Mechanisms in Postmetamorphic Molluscs

Two main drifting behaviours involving mucus have been observed in several molluscan taxa: (1) the production of large amounts of mucus originating from either the gills (*C. fluminea*; Prezant and Chalermwat 1984) or the foot (*Lacuna* spp.; Martel and Chia 1991a, 1991b) and (2) monofilamentlike mucous threads secreted by specialized glands situated in the foot (many marine bivalves, including *Mytilus*; Sigurdsson et al. 1976; Lane et al. 1982). In both types, drifting mucous threads act as a sea anchor or drag line by increasing the hydrodynamic drag and allowing resuspension in the water column with water currents. Active participation during the initial phase of post-metamorphic drifting in response to water currents has been demonstrated in some molluscs (genus *Lacuna*; Martel and Chia 1991b). In a study on the distribution and dispersal of the zebra mussel in the Great Lakes, Griffiths et al. (1991) mentioned that byssal threads are used as a means of drifting in juveniles, although no data were provided. Zebra mussels crawling and climbing on objects often produce long, stretchable mucous

threads secreted by the foot which are easily observed under a stereomicroscope using dark field. The involvement of mucous threads in drifting in zebra mussels has, however, yet to be verified (see Mackie 1991).

In addition to the possible use of monofilamentlike byssal threads during drifting, observations of zebra mussels found in plankton samples as well as in holding tanks with water currents suggest that two other drifting mechanisms may be involved: rafting and simple resuspension. Indeed, the frequent occurrence in plankton samples of early postmetamorphic stages with microscopic attachment byssus threads attached to detritus, fragments of adult byssus, or filamentous algae (e.g. *Cladophora*) indicates that juvenile drifting while rafting attached to fragments of substrate occurs in the field (A. Martel, unpubl. data). Zebra mussels attached to floating logs and debris as a means of dispersal in the Great Lakes has been mentioned by Mackie (1991); this could be accomplished by both juvenile and adult stages, the latter also capable of crawling over considerable distances (tens of centimetres in 24–48 h) (A. Martel, pers. obs.). Resuspension of early postmetamorphic stages lying on the bottom without any mucous threads or raft can also be observed when small individuals (generally under 1 mm) are placed on the bottom of a tank with turbulent water currents. This suggests that they may be resuspended if not attached (e.g. during crawling) or only weakly attached and dislodged by water currents in the field (see also Carlton (1993) for a review of natural and human-mediated dispersal mechanisms in the zebra mussel).

As in other molluscs where drifting occurs, sinking rates drastically increase with body size, making small juveniles (ideally under 1 mm) more likely to drift and remain in the water column due to turbulence (Lane et al. 1985; Martel and Chia 1991b). Sinking rates of early juvenile zebra mussels with shell lengths of 300–500 μm and placed in a still water column (25°C) are ~0.30–0.70 $cm \cdot s^{-1}$ only (no mucous thread involved) (A. Martel, unpubl. data). These sinking rates are comparable with those of certain anaesthetized crustacean zooplankton of a similar body length, such as *Daphnia schodleri*: ~0.25–0.40 $cm \cdot s^{-1}$ (see Hutchinson 1967). With such low sinking rates (unaided) for postmetamorphic zebra mussels, low-velocity upward water currents may be sufficient to keep postmetamorphic zebra mussels in suspension in the water column. Whether the drifting of postmetamorphic zebra mussels results from accidental dislodgement or involves an adaptive mechanism(s) that facilitates or enhances drifting (e.g. drifting mucous threads to reduce sinking rates) remains to be elucidated and will require detailed behavioural studies.

Impact on Colonization and Population Dynamics

Existing literature on the natural dispersal of zebra mussels and their colonization has focused on the role of planktonic veligers as the primary means of dispersal and recruitment (e.g. Stanczykowska 1977; Lewandowski 1982a, 1982b; Sprung 1989); I have demonstrated that drifting postmetamorphic stages of zebra mussels may also contribute significantly.

Previous studies have shown that postmetamorphic drifting plays an important role in the dispersal and colonization process of certain marine molluscs (Bayne 1964; Beukema and de Vlas 1989; Martel and Chia 1991a). Juvenile drifting also plays a similar role in the life history of certain brooding bivalves and gastropods that lack a planktonic larval stage (Martel and Chia 1991a). Drifting postmetamorphic recruits may have greater potential as colonists than settling larvae, as they have better chances of eventually reaching adulthood and contributing to reproduction. Juveniles arriving at a new habitat have already gone through the physiological stress of metamorphosis and the transition from planktonic to benthic life, a period during which mortality rates are believed to be very high (Lewandowski 1982b). In view of the high dispersal and colonization potential demonstrated by postmetamorphic stages, as shown by results presented here, it is noteworthy that the first zebra mussels to be detected (summer 1991) in the Kawartha Lakes area, Ontario (north of Lake Ontario), consisted of eight early juveniles (1–2 mm) collected in plankton samples from three different sites (L. Deacon, Ontario Ministry of Natural Resources, Lindsay, Ont., pers. comm.). Hence, the dispersal and colonization potential of postmetamorphic zebra mussels should be considered when studying or monitoring the natural spread of zebra mussels.

In areas subject to water currents or wave action (e.g. nearshore shallow waters, large lakes, rivers, water channels with barge traffic stirring up local currents, intake pipes), the drifting and relocation of high numbers of postmetamorphic stages may have a significant impact on patterns of recruitment of zebra mussels; this should be addressed in population studies. Colonization of natural or artificial substrates can, theoretically, occur not only during the general time of recruitment (i.e. summer), but also at times when zebra mussel veligers are usually absent in plankton (mid- to late fall, winter, early spring). In addition, new recruits can disappear from a habitat soon after metamorphosis, and the decrease in numbers be interpreted as mortality, when in fact relocation, via drifting, is also involved. Similarly, the arrival of young postmetamorphic recruits to a substrate may be interpreted as larval settlement, when in fact individuals colonized as drifting postmetamorphic stages. Finally, the drifting of such large numbers of early juveniles, as observed in the area of Wheatley during periods of high wave action, probably results in high mortality rates among drifters because a high proportion of them may not relocate to suitable habitats or microhabitats after sinking to their new locations. The common occurrence of empty shells 0.3–2 mm in length in plankton samples taken in the Wheatley area during periods of high wave action (A. Martel, unpubl. data) supports that idea of high mortality rates among small juveniles and corroborates results from previous European studies (Lewandowski 1982b).

Acknowledgments

I am most grateful to K. Klein for her generous support throughout the study, including assistance in field work and critical reading of the manuscript. R. Toonen helped in making the scouring pad collectors. D. Copp kindly provided laboratory assistance with numerous collector samples. J. Topping helped with the figures and, along with R. Chengalath, J. Fournier, T. Hynes, and two anonymous reviewers, provided many helpful comments on earlier versions of the manuscript. I would also like to thank the Director, S. Nepszy, and J. Leach, A. Matthew, and other staff of the Lake Erie Fisheries Station (Ontario Ministry of Natural Resources) at Wheatley for their most generous support while conducting field work. This research was supported by a Canadian Museum of Nature grant to A.M.

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Reduced Survival and Fitness in Native Bivalves in Response to Fouling by the Introduced Zebra Mussel (*Dreissena polymorpha*) in Western Lake Erie

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Haag, W. R., D. J. Berg, D. W. Garton, and J. L. Farris. 1993. Reduced survival and fitness in native bivalves in response to fouling by the introduced zebra mussel (*Dreissena polymorpha*) in western Lake Erie. Can. J. Fish. Aquat. Sci. 50: 13–19.

Fouling by the recently established zebra mussel (*Dreissena polymorpha*) causes differential effects among bivalve communities. In 3-mo experiments and surveys conducted in western Lake Erie during 1990, two native bivalves, *Lampsilis radiata* and *Amblema plicata*, showed consistent differences in mortality and biochemical indices of fitness in response to fouling by *D. polymorpha*. *Lampsilis radiata* was very sensitive to fouling, experiencing high mortality and reduced fitness in experiments and natural populations. In field experiments, female *L. radiata* suffered higher mortality and lower fitness than males. *Amblema plicata* was less sensitive to fouling; fitness was reduced in experimentally fouled *A. plicata*, but mortality and fitness in natural populations were not affected by *D. polymorpha*. Six species of native bivalves showed marked differences in mortality rates at three sites surveyed in western Lake Erie. Mortality was higher in the subfamilies Anodontinae and Lampsilinae (including *L. radiata*) than in Ambleminae (including *A. plicata*) at all three sites, suggesting that differences in life history strategy and shell morphology among subfamilies may be responsible for differential responses to fouling. These data suggest that fouling by *D. polymorpha* will result in profound changes in native bivalve community structure.

L'envahissement par des populations récemment établies de la dreissena polymorphe (*Dreissena polymorpha*) exerce différents effets sur les communautés indigènes de bivalves. Lors de relevés dans la partie ouest du lac Érie et d'expérience d'une durée de 3 mo, en 1990, on a montré que deux bivalves indigènes, *Lampsilis radiata* et *Amblema plicata*, manifestaient constamment des différences dans le taux de mortalité et dans les indicateurs biochimiques de santé par suite de l'envahissement par *D. polymorpha*. *Lampsilis radiata* était très sensible à cet envahissement : au cours d'expériences et dans des populations naturelles, la mortalité était élevée et la santé moins bonne. Au cours des expériences sur le terrain, la mortalité était plus élevée et la santé moins bonne chez les femelles que chez les mâles de *L. radiata*. *Amblema plicata* s'est révélé être moins sensible à l'envahissement : la santé était moins bonne chez les sujets de *A. plicata* expérimentalement soumis à un envahissement, mais la mortalité et la santé des populations naturelles n'ont pas été affectées par *D. polymorpha*. On a observé des différences marquées dans les taux de mortalité chez six espèces de bivalves indigènes à trois stations de la partie ouest du lac Érié. La mortalité était supérieure dans les sous-familles des Anodontinae et des Lampsilinae (y compris *L. radiata*) que chez les Ambleminae (y compris *A. plicata*) aux trois stations; cela pourrait signifier que des différences, entre les sous-familles, dans les cycle biologique ainsi que dans la morphologie des coquilles peuvent être à l'origine de réponses différentes à l'envahissement par *D. polymorpha*. Ces résultats indiquent que l'envahissement par *D. polymorpha* conduira à de profonds changements dans la structure de la communauté des organismes bivalves indigènes.

Received December 23, 1991
Accepted August 4, 1992
(JB347)

Reçu le 23 decembre 1991
Accepté le 4 août 1992

The sudden arrival of new species into established communities can result in massive restructuring because these communities evolved in the absence of, and hence have no mechanisms for dealing with, novel members. The introduction of nonnative aquatic organisms has had dramatic effects on native biota of the North American Great Lakes. Successful establishments of nonnative fishes, in particular, has dramatically altered native fish communites (Smith 1970; Christie 1974). The Eurasian zebra mussel (*Dreissena polymorpha*) has rapidly colonized the Great Lakes since its introduction in the mid-1980s, becoming a dominant member of the benthic fauna with densities exceeding 30 000 animals/m^2 in some areas (Griffiths et al. 1991). The sudden addition of *D. polymorpha* as an abundant member of the benthos has raised much concern about possible changes in benthic community structure in the Great Lakes.

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Bivalves of the family Unionidae are a diverse and conspicuous element of the benthic fauna of Lake Erie, with approximately 36 species native to the lake (Clarke and Stansbery 1988). Where common, unionids account for a large proportion of benthic biomass (Negus 1966) and serve as an important food resource for fish and other animals (Daiber 1952; Neves and Odum 1988). Unionids are infaunal filter-feeders, orienting themselves with the anterior end buried in the substrate and the posterior end (including the siphons) exposed to the water column. In contrast, *D. polymorpha* is a fouling epibiont, attaching to hard substrates with byssal threads much like marine fouling bivalves. Fouling is ecologically important in marine ecosystems, and many benthic marine organisms have developed elaborate antifouling strategies (Wahl 1989). In contrast, most freshwater communities have evolved in the absence of significant fouling organisms. The arrival of *D. polymorpha* in the Great Lakes represents the introduction of an important fouler into a community that has no evolutionary experience with, and therefore, no defensive mechanisms against, fouling. As a result, unionids and other benthic invertebrates often become heavily encrusted with aggregations of *D. polymorpha*. Individual unionids in Lake St. Clair have been reported to be encrusted with more than 10 000 *D. polymorpha* (Hebert et al. 1991). Such heavy encrustations may significantly interfere with the normal activities of unionids, such as filtering and locomotion (reviewed in Mackie 1991).

Because many populations of unionids have been severely reduced or extirpated due to habitat modification by humans, the appearance of *D. polymorpha* in North America has raised concern regarding its possible effects on already-stressed unionid populations (Hebert et al. 1989; Mackie 1991; Schloesser and Kovalak 1991). Several researchers have studied the effect of *D. polymorpha* on aquatic systems in Europe and North America, and some have speculated on its effects on other bivalves (Sebestyn 1938; Lewandowski 1975; Hebert et al. 1991; Mackie 1991). However, the impact of *D. polymorpha* fouling on survival and fitness in native unionids has not been thoroughly investigated and documented.

In this study, we examined the effects of fouling by *D. polymorpha* on native unionid bivalves. First, we used field experiments to test the hypotheses that (i) survival and biochemical indices of fitness in native unionids are reduced by *D. polymorpha* encrustation and (ii) different species of unionids show different responses to fouling. Then, we examined mortality and fitness as a function of the number of encrusting *D. polymorpha* in natural populations of the two unionid species used in the field experiment. Finally, we surveyed patterns of recent mortality among six unionid species in natural populations in western Lake Erie.

Methods

Experiment

Adult unionids of two species, *Lampsilis radiata* and *Amblema plicata* (55–85 mm in length), were collected from large natural populations in Lake Erie at Put-in-Bay and Kelleys Island, OH (approximately 41°38′N, 82°50′W and 41°34′N, 82°42′W, respectively), in early July 1990. Attached *D. polymorpha* were removed from every unionid, and each unionid was measured and weighed. Each species was randomly divided into two groups (experimental and control) of approximately 40 animals. For *L. radiata*, both groups contained approximately equal numbers of males and females, as this species can be sexed externally. Female specimens of *L. radiata* have inflated and elongated valves accommodating gills with enlarged marsupia during the brooding of glochidia; male specimens lack this characteristic. *Amblema plicata* is not sexually dimorphic and cannot be sexed from external shell morphology. Therefore, sex ratio was not controlled for *A. plicata*. For both unionid species, the control group was left cleared of *D. polymorpha*. Experimental (i.e. encrusted) animals were placed in aerated flow-through aquaria, allowed to orient themselves naturally in sand, and then covered with a layer of *D. polymorpha* scraped from rocks collected nearby. Zebra mussels were allowed 24–48 h to attach to the unionids. This technique resulted in dense encrustations similar to natural conditions. Mean number of *D. polymorpha* on experimental animals was 199.5 ± 19.0 (SE, $n = 27$), while mean number on naturally encrusted animals was 215.9 ± 14.5 (SE, $n = 92$). Although the size range of encrusting *D. polymorpha* on each experimental animal could not be controlled, the zebra mussel mass on reencrusted unionids appeared very similar to field-collected encrusted unionids. To aid identification and recovery, experimental and control animals were marked by scribing shallow grooves in the anterior end of the right or left valve, respectively.

Approximately 2 wk after collection, all animals were returned to the lake and placed in 4-m^2 open-topped chicken-wire pens at a depth of 1.75 m at Put-in-Bay, OH. The large diameter of the chicken wire (25 mm) and the open top of the pens ensured that water flow was not restricted in the pens, which merely served to facilitate recovery of the animals at the end of the experiment. Pens were inspected 2 wk after initiation of the experiment to remove dead animals that may have been stressed during holding in the laboratory. Animals that died during this period were not considered in later data analyses. During this period, four control and two experimental specimens of *L. radiata* died; no specimens of *A. plicata* died.

In late October 1990, all animals were retrieved and mortality was recorded. Over 80% of marked animals for each species–treatment combination were recovered. Control animals remained relatively free of *D. polymorpha* throughout the experiment (mean number of *D. polymorpha* on controls at the end of the experiment was 3.3 ± 0.8 (SE)). Survivors were wet weighed and soft tissues were flash frozen for later biochemical analyses. Soft tissues of unionids were homogenized and divided into four measured aliquots for determination of total glycogen and lipid content, cellulase enzyme activity, and estimated dry weight. Glycogen content was determined using a phenol – sulfuric acid method (MacInnis and Voge 1970) and total lipid content using a vanillin – phosphoric acid technique (Van Handel 1985). Cellulase activity, which has been shown to be negatively correlated with stress in bivalves, was determined using an assay described by Farris et al. (1988). Biochemical indices were weight standardized using the general form $V_x = aW^b$, where V_x is total glycogen, lipid, or enzyme activity for the unionid, W is total wet or dry weight, and a and b are fitted constants. The constants a and b are calculated from the linear regressions of the log V_x on the log of body mass; a represents the intercept and b the slope of the regression equation. Weight-specific rate variables were finally calculated as $\dot{V}_x = V_x/W^b$ where $\dot{V}_x$ is the weight-specific biochemical index and expressed as milligrams and micrograms per gram wet weight (glycogen and lipid) or units per microgram dry weight (cellulase activity) per standard animal (in this case, the average wet or dry weight for all specimens of that species). Statistical differences in mortality and weight-specific

biochemical indices of fitness among groups were tested using analysis of variance (ANOVA).

Survey

Lampsilis radiata and *A. plicata* 55–85 mm in length were collected from natural populations at Put-in-Bay, Kelleys Island, and LaPlaisance Bay, MI (approximately 41°50′N, 83°22′W), in October 1990, within 2 d of termination of the field experiments. Unionid specimens were hand-collected randomly using SCUBA. *Amblema plicata* was common at all three sites, but *L. radiata* was common only at Put-in-Bay, and sample sizes for Kelleys Island and LaPlaisance Bay were small. Animals were weighed, and prepared for biochemical assays following the same procedure used for experimental animals. Total number of encrusting *D. polymorpha* was determined for each unionid, and weight-specific glycogen and lipid content and cellulase activity were correlated with number of *D. polymorpha* using regression and analysis of covariance (ANCOVA).

In October 1990, native bivalve communities at the three sites in western Lake Erie described above were surveyed for suvivorship of six common species belonging to three subfamilies, *Anodonta grandis* (Anodontinae), *L. radiata, Leptodea fragilis,* and *Potamilus alatus* (Lampsilinae), and *Quadrula pustulosa* and *A. plicata* (Ambleminae). Specimens of live and dead unionids were hand-collected using SCUBA. All six species were present at each site, but only species for which five or more individuals were collected were included in data analyses. All living and recently dead unionids were collected, enumerated, and scored as either encrusted or not encrusted. Only encrusted animals were included in mortality data analysis. Because the nacrous layer of bivalve shells remains lustrous for several months after death, animals that have died recently can be identified (Buchanan 1980). A high proportion of live to recently dead animals for a given species was interpreted as a low mortality rate within the past year, while a low proportion of live animals was interpreted as high mortality. Differences in proportion of live to dead animals among subfamilies and species were tested by calculating chi-square statistics from R × C contingency tables or Fisher's exact test if tables had low expected values.

Results

Experiment

Dreissena polymorpha encrustation produced species-specific and sex-specific mortality in the study species (Table 1). No mortality was observed among *A. plicata* in either the experimental or control group. In *L. radiata*, however, encrusted females experienced much lower survival (16.7%) than control animals (68.4% for both males and females) or encrusted males (64.3%) (G-test, $p < 0.01$). There were no differences in survival between male and female control animals or between male experimental and control animals. Biochemical indices of fitness also differed for the two species in response to *D. polymorpha* encrustation. Although encrusted specimens of *A. plicata* experienced no mortality, mean glycogen content and cellulase activity was significantly lower in encrusted animals than in controls (Table 1). Lipid content was not significantly different between the two groups. Surviving specimens of *L. radiata* showed sex-specific responses to encrustation (Table 1). Mean glycogen content and cellulase activity were significantly lower in encrusted animals than in controls, and females had lower glycogen content and cellulase activity than males. There were no differences in lipid content among treatment or sexes in specimens of *L. radiata*.

Survey

Mortality in natural populations of *L. radiata* and *A. plicata* reflected experimental results. Proportions of live to dead animals were lower for *L. radiata* than for *A. plicata* at the two sites having sufficient numbers of *Lampsilis* for analysis (Table 2). At LaPlaisance Bay, MI, *L. radiata* was too rare for inclusion in this analysis. Mortality was low for *A. plicata* in the western basin, with greater than 90% of animals alive at all three sites, whereas 66% of *L. radiata* were collected live (Table 2).

Correlations of fitness and number of encrusting *D. polymorpha* differed among specimens of *A. plicata* and *L. radiata* from natural populations. For *A. plicata* at all three sites, there were no significant relationships between glycogen, lipid content, or cellulase activity and total number of encrusting *D. polymorpha* (Fig. 1). Sufficient specimens of *L. radiata* for biochemical analyses were collected only at Put-in-Bay. There were no between-sex differences in the three biochemical measures, so sexes were pooled for futher analyses. Glycogen content was negatively correlated with number of encrusting *D. polymorpha* (Fig. 2). Lipid content and cellulase activity were not correlated with number of *D. polymorpha* by simple linear regression. However, combining data sets from experimentally and naturally encrusted animals in an ANCOVA (with experimental group and field population as the treatment variables) and using number of *D. polymorpha* as a covariate revealed a significant negative correlation between cellulase activity and number of encrusting zebra mussels.

Biochemical indices of fitness in populations of *A. plicata* and *L. radiata* showed large interpopulation differences unrelated to the density of encrusting *D. polymorpha* (Table 3). Although within-sample variances were high, sample sizes were adequate for parametric analyses for *A. plicata* ($n = 15$ for Put-in-Bay and LaPlaisance Bay and $n = 9$ for Kelleys Island). However, sample sizes of specimens of *L. radiata* were less balanced, with $n = 4$ for LaPlaisance Bay and Kelleys Island and $n = 27$ for Put-in-Bay. Therefore, a nonparametric analysis was used for final analysis of biochemical indices for populations of *L. radiata*, which successfully equalized within-sample variance for all populatons. Identical results were obtained for parametric ANOVA and nonparametric Kruskal–Wallis k-sample tests for all three biochemical indices for specimens of *A. plicata* and differed only for cellulase activity for specimens of *L. radiata*. Mean glycogen content did not differ between specimens from Put-in-Bay and Kelleys Island for both species. Mean cellulase activity was higher in specimens of *L. radiata* from Put-in-Bay than in those from Kelleys Island. Lipid content was generally low in both species. Lipids were slightly lower in specimens from Kelleys Island for both species, with significant differences between populations at Kelleys Island and Put-in-Bay for *A. plicata* and between those at Kelleys Island and LaPlaisance Bay for *L. radiata*.

Patterns of mortality in natural populations of unionids in Lake Erie varied across species, but were taxonomically consistent at the subfamily level. Proportions of live to dead animals were lower for the subfamilies Anodontinae (*A. grandis*) and Lampsilinae (*L. radiata, L. fragilis,* and *P. alatus*) than for Ambleminae (*A. plicata* and *Q. pustulosa*) at all three sites (Table 2). At Put-in-Bay, proportions of live to dead animals for the Anodontinae were lower than for Lampsilinae, but did

TABLE 1. Survival, glycogen and lipid content, and cellulase enzyme activity for specimens of *A. plicata* and *L. radiata* in the field experiment. Values in parentheses represent standard errors of the means. Glycogen and lipid content are based on tissue wet weights, while enzyme activity is based on tissue dry weights.

Species	Treatment	n^a	% Survival	Glycogen content (mg/g)	Lipid content (μg/g)	Enzyme activity (units/μg)	n^b
Amblema plicata	Control	34	100 (—)	1.90 (0.23)	2.2 (0.1)	257.4 (24.9)	15
	Encrusted	35	100 (—)	0.67 (0.20)	2.0 (0.1)	87.8 (16.0)	15
Lampsilis radiata							
Male	Control	19	68 (11)	0.13 (0.02)	2.7 (0.1)	399.6 (62.3)	13
	Encrusted	14	64 (11)	0.08 (0.01)	2.7 (0.1)	151.6 (28.4)	9
Female	Control	19	68 (11)	0.08 (0.01)	3.0 (0.1)	200.5 (44.3)	13
	Encrusted	18	17 (9)	0.04 (0.01)	3.7 (0.3)	111.5 (27.2)	3

[a]Number of individuals placed in field enclosures at start of experiment.
[b]Number of animals used for biochemical analyses.

TABLE 2. Ratio of live individuals to dead individuals of unionid species from three sites across western Lake Erie. Proportion alive for *L. radiata* is significantly lower than for *A. plicata* (Fisher's exact test, $p < 0.01$). Proportion alive for Anodontinae and Lampsilinae is significantly less than for Ambleminae for all sites pooled (goodness of fit test, $p < 0.001$).

	Location				
Subfamily and species	LaPlaisance Bay	Put-in-Bay	Kelleys Island	Total	Proportion alive
Anodontinae					
Anodonta grandis	6:10	1:13	2:4	9:27	0.25
Lampsilinae					
Leptodea fragilis	7:11	2:5	—[a]	9:16	0.36
Potamilus alatus	24:10	3:8	1:4	28:22	0.56
Lampsilis radiata	—[a]	48:19	2:7	50:26	0.66
Ambleminae					
Quadrula pustulosa	34:3	—[a]	7:0	41:3	0.87
Amblema plicata	29:3	44:2	9:1	82:6	0.93

[a]Insufficient number of individuals ($n < 5$) collected for inclusion in analyses.

not differ at Kelleys Island or LaPlaisance Bay. At all three sites, proportions of live to dead animals were high for the subfamily Ambleminae, indicating that these species had not suffered recent mortality. It must be noted that there are no similar data from Lake Erie prior to the introduction of *D. polymorpha* with which to compare our observations. However, the taxonomic and geographic consistency of our observations suggest that strong, differential mortality in unionid populations had occurred across western Lake Erie within the previous year.

Discussion

Effects on Individual Species

Fouling by *D. polymorpha* has profound but variable effects on unionid species in western Lake Erie. Some species (e.g. *L. radiata*) may suffer dramatic reductions in fitness and increased mortality due to fouling by *D. polymorpha*. Other species (e.g. *A. plicata*) are not significantly affected in the short term, but fouling significantly reduces long-term fitness by decreasing glycogen content and increasing stress. Survey results corroborate experimental results. Natural populations of *L. radiata* experienced higher mortality in the western basin of Lake Erie in the recent past, following the arrival of *D. polymorpha*, than *A. plicata*. Thus, two common species in the Lake Erie bivalve community are differentially affected by the presence of *D. polymorpha*, with *A. plicata* being more tolerant of fouling than *L. radiata*.

Biochemical indices of fitness were more variable in encrusted animals from natural populations than in animals from the experiments, but these data are consistent with the hypothesis that *A. plicata* is more tolerant of fouling than *L. radiata*. Concordant with experimental results, glycogen content and cellulase activity, but not lipid content, were negatively correlated with *D. polymorpha* encrustation in natural populations of *L. radiata*. In contrast with experimental results, specimens of *A. plicata* collected in the field survey suffered no detectable reduction of biochemical fitness as a function of *D. polymorpha* encrustation. The number of encrusting *D. polymorpha* was variable on animals collected from natural populations, but most animals were heavily encrusted. Our experiments, however, consisted of heavily encrusted animals compared with completely cleared individuals. Consequently, a relatively tolerant species such as *A. plicata* may show short-term effects of fouling only in strictly controlled situations, while a less tolerant species such as *L. radiata* could show high mortality and observable reductions in fitness in response to fouling despite the confounding variables of an uncontrolled field situation. Thus, although significant effects can be induced in controlled experiments, the effects of *D. polymorpha* fouling in natural populations of unionids are likely to be more subtle and variable.

In bivalves, glycogen is the primary energy storage molecule (often present in excess of 30% of total body dry weight) and

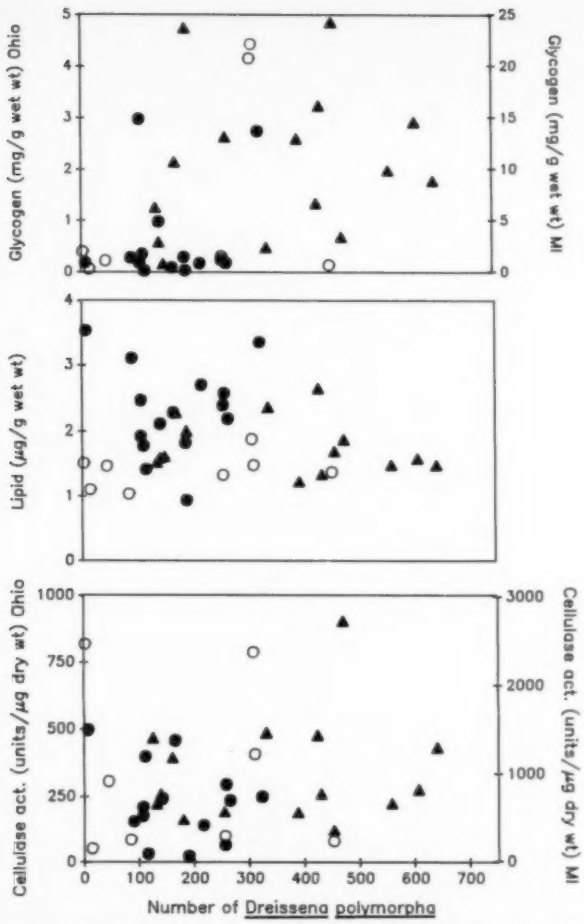
Glycogen (mg/g wet wt) Ohio
Glycogen (mg/g wet wt) MI
Lipid (µg/g wet wt)
Cellulase act. (units/µg dry wt) Ohio
Cellulase act. (units/µg dry wt) MI
Number of Dreissena polymorpha

FIG. 1. Biochemical indices of fitness in *A. plicata* from three natural populations in western Lake Erie. Filled circles represent animals from Put-in-Bay, OH, open circles represent animals from Kelleys Island, OH, and triangles represent animals from LaPlaisance Bay, MI. Horizontal axes represent the number of encrusting *D. polymorpha*. There were no significant correlations between biochemical indices of fitness and number of encrusting *D. polymorpha*.

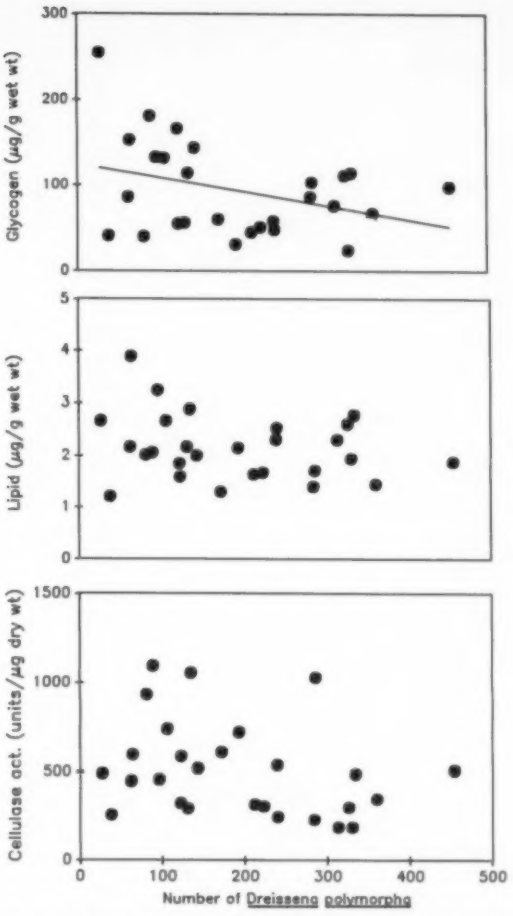

FIG. 2. Biochemical indices of fitness in *L. radiata* from a natural population in Lake Erie at Put-in-Bay. OH. Glycogen content was negatively correlated with number of encrusting *D. polymorpha* ($r^2 = 0.23$, $n = 27$, $p < 0.03$).

lipid reserves are generally low (reviewed in Pandian 1975). In this study, glycogen content was 1–2 orders of magnitude higher than lipid content for both *L. radiata* and *A. plicata*. Lipid content was less than 1.29% of body weight (not reported whether based on wet or dry weight) for Lake St. Clair unionids (Hebert et al. 1991). Our field experiments revealed that glycogen content was sensitive to fouling by *D. polymorpha* whereas lipid content did not differ between encrusted and unencrusted animals. Similarly, glycogen content was inversely related to number of encrusting *D. polymorpha* for specimens of *L. radiata* from a natural population, while lipid content was not related to encrustation density. Thus, fouling by *D. polymorpha* results in reductions in energy storage (reduced glycogen content) and higher stress (lower cellulase activity), but not necessarily in reduced content of primarily structural molecules such as lipids. In contrast with these results, Hebert et al. (1991) concluded that fouling by *D. polymorpha* causes a reduction in lipids in Lake St. Clair unionids, although no regression statistics were provided to support the assertion. Although it is plausible that encrustation by *D. polymorpha* may ultimately reduce lipid content, significant reductions in the primary energy store, glycogen, will have more serious and immediate effects on survival and reproduction of native unionids.

Mean glycogen and cellulase activity in native unionids varied across sites in western Lake Erie, independent of encrustation densities of *D. polymorpha*. Lipid content also varied significantly across sites, but the differences were less dramatic. In general, unionids collected at Kelleys Island had lower lipid content than those at the other two sites. Local environmental conditions (e.g. temperature regimes, quantity and quality of food, substrate stability, etc.) may have strong effects on the nutrient status of native unionids. Although parameters of environmental quality at the three collecting sites were not measured in this study, consistent patterns of biochemical indices among sites suggest significant differences in habitat quality across western Lake Erie. Preliminary studies of the impact of *D. polymorpha* on unionids have pooled samples across sites without considering possible site differences in baseline nutritional status (e.g. Hebert et al.

TABLE 3. Weight-standardized glycogen content, lipid content, and enzyme activity for *A. plicata* and *L. radiata* from three natural populations in western Lake Erie. Site abbreviations: PIB = Put-in-Bay, OH; KEL = Kelleys Island, OH; MI = LaPlaisance Bay, MI. Values are means with standard errors in parentheses. Species means within the same column with the same superscript letter are not significantly different; those with different supercripts are significantly different (Tukey's multiple comparison for *A. plicata*; Kruskal–Wallis *k*-sample test for *L. radiata*). Glycogen and lipid contents are based on tissue wet weights, while enzyme activity is based on tissue dry weights.

Species and site	Glycogen content (mg/g)	Lipid content (μg/g)	Enzyme activity (units/μg)	*n*
Amblema plicata				
PIB	0.06 (0.24)[a]	2.30 (0.18)[a]	357.0 (135.0)[a]	15
KEL	1.71 (0.75)[a]	1.38 (0.09)[b]	328.5 (99.2)[a]	9
MI	10.36 (1.86)[b]	2.01 (0.26)[a,b]	1010.0 (156.0)[b]	15
Lampsilis radiata				
PIB	0.09 (0.01)[a]	2.15 (0.12)[a,b]	512.0 (51.6)[a]	27
KEL	0.05 (0.01)[a]	1.40 (0.21)[b]	72.1 (21.7)[b]	4
MI	7.62 (5.35)[b]	2.76 (0.33)[a]	1168.0 (347.0)[a]	4

TABLE 4. Standardized left valve weight for six native bivalve species. Adjusted weight is calculated for a standard 78.2-mm valve from linear length–weight regressions of nontransformed data for each species. Increasing slopes of regression equations indicate thicker shells. Regressions were determined for samples from western Lake Erie, except for *Q. pustulosa* which is from the Muskingum River, OH. Values in parentheses are standard errors of the means.

Subfamily	Species	*n*	Shell length (range, mm)	Slope	Adjusted left valve weight (g)
Ambleminae	*Quadrula pustulosa*	7	23.5– 69.9	1.19	59.55 (1.52)
	Amblema plicata	8	62.9– 74.0	0.84	34.22 (1.85)
Lampsilinae	*Lampsilis radiata*	8	60.2– 81.7	0.63	20.85 (1.10)
	Potamilus alatus	8	68.0–109.4	0.64	14.36 (0.83)
	Leptodea fragilis	7	88.7–111.3	0.42	7.83 (2.09)
Anodontinae	*Anodonta grandis*	8	72.6–131.0	0.51	4.63 (0.81)

1991). Clearly, field studies assessing the potential impacts of encrustation by *D. polymorpha* on biochemical nutrient status of native unionids must account for potential geographic variation in environmental quality.

Unionids feed by filtering small particles from the water column or water – sediment surface interface. Feeding by unionids may be impaired by encrustation and/or loss of suspended particles to grazing *D. polymorpha*. Species and sex-dependent behavioral and morphological differences may have contributed to differential responses to fouling by *D. polymorpha*. Individual unionids which position the posterior margin of the shell further above the substrate, or which have longer siphons, may have better access to available food in the water column. However, differences in feeding behavior and siphonal morphology among species or between sexes have not been studied, and in general, the feeding biology of many species of unionids is not well known (Clarke 1981).

Effects on Bivalve Community Structure

Experimental and survey data indicate that the introduction of *D. polymorpha* into the Great Lakes will alter the community structure of unionid bivalves by causing differential mortality and reductions in fitness among some unionid species. Similarly, the introduction of the marine bivalve *Potamocorbula amurensis* into San Francisco Bay resulted in the displacement of the native bivalve community (Nichols et al. 1990). Other nonnative freshwater bivalves have become established in North America (notably *C. fluminea*; see McMahon 1983), but none has been shown to result in extreme and rapid changes in community composition (Leff et al. 1990). *Corbicula fluminea* is similar to native bivalves in its habit of burrowing into the substrate and thus becomes simply another species in an already species-rich community. Accordingly, *C. fluminea* may be unable to become established in areas with healthy unionid populations because the niche of infaunal, filter-feeding bivalve is already filled (reviewed in McMahon 1983). Epibiotic *D. polymorpha*, however, present a novel challenge to freshwater communities that are naive to fouling. Thus, *D. polymorpha* is easily able to fill the previously empty niche of the epibiont, and effects of fouling are variable among different species of unionids which evolved different life histories, habitat preferences, and other traits in the absence of fouling organisms.

Potential mechanisms for the negative effects of *D. polymorpha* on native unionids have been proposed (Mackie 1991): (i) encrusting biomass hinders locomotion and burrowing, (ii) encrusting biomass concentrated at posterior end disrupts balance and equilibrium, (iii) invasive growth by zebra mussels prevents normal closure and/or opening of valves, (iv) interference of normal functioning of siphons, (v) filter feeding by zebra mussels strips water of available food, (vi) deformation of unionid valves, and (vii) complete valve occlusion leads to suffocation. Any combination of these mechanisms may contribute to unionid mortality or reduction in biochemical fitness.

Most of the above hypotheses have yet to be tested directly, but consistent results within subfamilies in response to fouling by *D. polymorpha* suggest likely mechanisms responsible for differential mortality and reduction in fitness in Lake Erie

unionids. The Anodontinae and Lampsilinae have experienced widespread mortality as *D. polymorpha* has become an abundant member of the benthos in western Lake Erie, while the Ambleminae have not. The Anodontinae and Lampsilinae share a common trait of females brooding glochidia larvae in the gills for long periods, usually 9–12 mo (Clarke 1981). Ambleminae, however, are short-term breeders, brooding young for only a few weeks to 2 mo. Therefore, female anodontines and lampsilines invest considerably more time, and presumably energy, into brooding of larvae (Mackie 1984) and in this way may be more sensitive to fouling than amblemines; also, male lampsilines and anodontines are expected to be less sensitive than females to fouling because they do not brood larvae. As predicted by this hypothesis, our data show severe mortality for fouled female *L. radiata* but no difference in mortality between encrusted and unencrusted males, and no mortality for the short-term breeder, *A. plicata*. Thus, a long-term strategy which evolved in a pre-*D. polymorpha* environment may now be disadvantageous in an environment with abundant fouling organisms.

Anodontinae and Lampsilinae also differ from the Ambleminae in shell morphology. Amblemines are characterized by solid, heavy shells whereas anodontines and lampsilines typically have much lighter shells (Table 4). Like many bivalves, unionids must maintain specific orientation in the substrate for efficient filter feeding and waste excretion. The addition of a large mass of *D. polymorpha* to the posterior surface of a light-shelled anodontine or lampsiline will change the center of gravity for the animal and make it more energetically expensive to maintain proper posture in the substrate. For lampsilines and anodontines in western Lake Erie, the weight of fouling *D. polymorpha* on an individual unionid may exceed the weight of the unionid by a factor of 4 (Schloesser and Kovalak 1991). In contrast, the center of gravity of a heavy-shelled amblemine would be less affected by additional weight on the shell. Again, as predicted by this hypothesis, heavy-shelled amblemines show low mortality rates as compared with lampsilines and anodontines. Heavy-shelled species may also have stronger adductor muscles and hence be less vulnerable to invasive growth by *D. polymorpha* within the gape. Thus, differences in shell morphology among unionid subfamilies may result in variable responses to fouling by *D. polymorpha*.

The introduction of *D. polymorpha* into the Great Lakes has had profound and rapid effects on the structure of native bivalve communities. The community in Lake Erie can be expected to move increasingly towards domination by thick-shelled amblemines as lampsilines and anodontines are removed by short-term mortality and reductions in fitness. However, amblemines may also experience an overall decline in abundance in the long term because these species are more slowly and subtly affected by heavy fouling by *D. polymorpha*. The lack of any evolutionary experience with fouling in North American unionids means that suites of characters that have arisen in a freshwater environment will suddenly be subject to entirely new selection regimes. In Lake Erie bivalve populations, differences in reproductive strategy, shell morphology, and other factors may suddenly be manifested as differences in responses to fouling by *D. polymorpha*.

Acknowledgements

This project would not have been possible without the assistance of John R. Hageman, manager of The Ohio State University's F. T. Stone Laboratory at Put-in-Bay, OH. Jack Wade, Paul Buescher, and other members of the Ohio Underwater Research Association assisted in the collection of animals from Kelleys Island. We would also like to thank R. Hancock, P. Pappas, T. Cvetnik, P. Berg, L. Hall, B. Ling, D. Jameson, and D. Insley for assistance in the laboratory and field. D. Stansbery of the Ohio State University Museum of Zoology provided unionid specimens for measurement of shell weight. S. Madon and C. Franz critically reviewed early drafts of the manuscript, and comments from H. MacIsaac and an anonymous reviewer on the final draft were very useful. This project was supported by the Ohio Sea Grant Development Fund and American Electric Power Company.

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Spawning Mode and Reproductive Output of the Tropical Cephalopod *Idiosepius pygmaeus*

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Lewis, A. R., and J. H. Choat. 1993. Spawning mode and reproductive output of the tropical cephalopod *Idiosepius pygmaeus*. Can. J. Fish. Aquat. Sci. 50: 20–28.

Reproductive biology of the tropical sepioid cephalopod *Idiosepius pygmaeus* was investigated in wild specimens and in individuals maintained in aquaria through the adult life span. This species produced multiple egg batches over 80% of the observable adult weight range, indicating a coordination of reproductive and somatic growth. Reproductive output was consistent within, but variable between, individuals. Oocyte synthesis and maturation occur continuously after sexual maturation has been reached. Senescence and death are not related to an exhaustion of reproductive potential. On average, captive female *I. pygmaeus* with access to unlimited food produced 640 eggs in 11 batches over 18 d. When reproductive output was expressed as a ratio of dry female body weight, on average, specimens had incorporated five times their body weight into eggs and egg coatings. Under food stress, captive specimens laid fewer eggs but maintained egg size and periodicity of egg laying. Food stress had no effect on either laying duration or weight at death. This study provides further evidence that terminal spawning modes are not ubiquitous amongst cephalopods.

Chez le céphalopode tropical sépioïde *Idiosepius pygmaeus*, la biologie de la reproduction a été étudiée sur des sujets à l'état sauvage et des sujets maintenus dans des aquariums pendant toute la durée de leur vie adulte. Chez cette espèce, les pontes peuvent correspondre à plus de 80 % de l'échelle observable des poids chez les adultes; cela est révélateur d'une coordination entre la croissance somatique et la croissance associée à la reproduction. Sur le plan de la reproduction, la production était uniforme chez un même sujet, mais variait d'un sujet à l'autre. La synthèse et la maturation d'ovocytes ont lieu sans interruption après que la maturation sexuelle ait été atteinte. La sénescence et la mort ne sont pas associées à un épuisement du potentiel reproductif. En moyenne, les femelles captives de *I. pygmaeus* qu'on alimentait à satiété produisaient 640 oeufs en onze pontes sur un intervalle de 18 jours. Lorsque la production d'oeufs était exprimée en proportion du poids sec des femelles, on constatait qu'en moyenne, les sujets avaient incorporé dans les oeufs et l'enveloppe des oeufs l'équivalent de cinq fois leur masse corporelle. Les sujets captifs qui étaient sous-alimentés pondaient moins d'oeufs, mais le volume de chaque oeuf et la périodicité de la ponte restaient constants. La sous-alimentation n'a pas eu d'effet ni sur la durée de la ponte ni sur le poids à la mort. Cette étude vient confirmer à nouveau que ce ne sont pas tous les céphalopodes qui se comportent selon un mode de ponte terminal.

Received April 21, 1992
Accepted July 17, 1992
(JB476)

Reçu le 21 avril 1992
Accepté le 17 juillet 1992

Coleoid cephalopods (squids and cuttlefish) are considered to have a terminal reproductive mode (Arnold and Williams-Arnold 1977; Arnold 1984; Mangold 1987; Heller 1990). Most species produce single or few egg batches, with spawning signalling a decline in growth and the onset of senescence and death (Bergstrom and Summers 1983; O'Dor 1983; Okutani 1983; Forsythe and Van Heukelem 1987; Boyle 1990). The notion of terminal spawning is central to most models of cephalopod life history biology (O'Dor and Webber 1986; Calow 1987; Vecchione 1987).

Harman et al. (1989) found evidence to suggest that egg laying in the tropical ommastrephid squid *Stenoteuthis oualaniensis* may be protracted and that growth occurs between spawning events. Rodaniche (1984) also documented multiple spawning in conjunction with growth in the small tropical octopod *Octopus chierchiae*. These studies indicate that models of cephalopod life history biology may need revision, yet no detailed schedules of age- or size-specific reproductive output exist for multiple-spawning tropical cephalopods. In addition, no workers have expressed cephalopod fecundity as a proportion of maternal somatic mass or energy content, considered important if meaningful estimates of reproductive output are to be made (Thompson 1982).

A difficulty in the study of reproductive events in coleoid cephalopods is the requirement of obtaining estimates of the distribution and magnitude of output over the adult life span. This usually requires maintenance in culture for relatively long periods and ideally a parallel study program of reproductive anatomy and the dynamics of oocyte production.

The small sepioid squid *Idiosepius pygmaeus* can be found in nearshore waters off Townsville, North Queensland. Details of the life history are provided in Jackson (1989). The ease with which this species may be kept in captivity and the short (<80 d) life span provide an ideal opportunity to collect information regarding fecundity and other aspects of reproductive biology from many specimens over their reproductive life span. Previous investigations of the reproductive output of *I. pygmaeus*, although conducted on less than 10 specimens, suggest that this species is capable of producing multiple egg batches and in relative terms is likely to have high fecundity (Natsukari 1970; Jackson 1991).

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The aim of this study was to obtain estimates of the life-time fecundity of *I. pygmaeus* particularly with regard to rates, variation, and periodicity of egg production. Two approaches have been taken. Firstly, *I. pygmaeus* were maintained under conditions of high and low food availability to provide the essential fecundity estimates. Secondly, this was linked to a program of anatomical and histological analysis of the reproductive system in order to correlate egg output rates with the internal dynamics of oocyte production in specimens maintained in captivity and from field collections.

Materials and Methods

Collection Methods and Laboratory Conditions

Idiosepius pygmaeus were collected with a dip net between March and June 1991 from close to the water surface adjacent to rock breakwaters and mangroves near Townsville, North Queensland, Australia (19°15′S, 146°50′E). Upon capture, each specimen was placed in a quick-seal plastic bag (25 × 15 cm) and transferred to aquaria usually in less than 2 h. No deaths occurred during this phase of collection.

Female *I. pygmaeus* were maintained individually within sixteen 30-cm^3 aquaria attached to a 160 000-L recirculating seawater system. Aquaria were sited outdoors to ensure normal diel periodicity but were covered with semitransparent plastic sheeting to prevent water temperature from becoming excessively high. Rocks and shells were provided as shelter for the animals and as sites for egg attachment. As captive females died of senescence, immature sepioids were collected from the field as replacements. Specimens were fed live sergestid shrimp (*Acetes sibogae australis*) (5–12 mm total length), which was the observed prey in the field.

Female *I. pygmaeus* are solitary in the field (pers. obs.) and have the ability to store sperm (Mangold 1987). For these reasons, male *I. pygmaeus* were introduced to the individual female aquaria only once per week for mating purposes. Mating was initiated immediately by the male in a head to head position.

Egg Collection and Processing

Data regarding reproductive output were collected from 50 female *I. pygmaeus* maintained in captivity during the 107-d period March 9 to June 24, 1991. Water temperature decreased from 27.5 to 23°C over this period.

Aquaria were usually checked twice daily (09:00 and 17:00) for eggs, which were laid in a gelatinous mass. The egg mass was removed from its point of attachment using a razor blade and a pipette (bore 5 mm). Individual eggs were counted and then the entire mass was transferred to a preweighed 5-mL plastic vial for freeze-drying using a Dynavac FD6 machine. Weights of freeze-dried eggs, together with all other weight measurements in this study, were recorded to the nearest 0.1 mg using a Sartorius 2004 MP electronic balance.

Eggs of *I. pygmaeus* are laid surrounded by gel coatings which swell via hydration upon contact with seawater. In order to determine what proportion of the freeze-dried weight of the egg batch was due to organic material (as opposed to salt incorporated during hydration), all egg batches from seven captive females, including specimens from both feeding regimens, were ashed at 600°C in a high-temperature furnace for 6 h. After this time, no further reductions in weight occurred; hence, weight loss after 6 h was taken to equal the organic content of the egg batch. Ashing dry eggs at this temperature will oxidise all nonmetallic elements of low molecular weight (i.e. H, C, N, O), leaving behind heavier metallic and halide elements such as Na and Cl. Metallic elements incorporated into eggs by *I. pygmaeus*, such as K and various trace elements, will not be removed; hence, the values of organic weight derived from the ashing process should be considered underestimates. Egg batches for ashing were selected to represent a large range of weights and weights per egg from females of both feeding regimes; random selection was not employed.

To determine the relationship between wet and dry weight, 22 freshly killed *I. pygmaeus* from 47.6 to 618.3 mg were weighed (after being blotted dry with paper towel) and then freeze-dried on the Dynavac FD6 and the dry weight recorded.

Preservation Methods and Histological Techniques

Both wild-caught sepioids and individuals from the captive study with known feeding and reproductive histories were preserved for histological examination. Animals were narcotised in a weak alcohol solution, measured (dorsal mantle length) using an Olympus SZ 40 binocular microscope equipped with a micrometer eyepiece, weighed (wet weight), and then transferred directly to a solution of formalin (10%), acetic acid (5%), and calcium chloride (1.3%) by volume (*faacc*). Specimens were fixed in at least 10 times their body volume of *faacc* for 2–7 d and transferred to a 70% ethanol solution (to prevent excessive tissue hardening) for at least 24 h before processing.

Specimens were processed to paraffin wax for sectioning. Small specimens could be embedded whole and sectioned longitudinally. Larger sepioids were cut with a razor blade into 3-mm transverse blocks prior to embedding. Each block was then embedded and sectioned transversely. Sections were cut at 6 μm on a rotary microtome and were stained with Mallory-Heidenhain Trichrome stain (Winsor 1984).

Photomicrographs were taken using an Olympus PM-10 35-mm camera and an Olympus BH-2 photomicroscope. Negatives were made on Ilford Pan-F 50 ASA film using a green filter to enhance contrast.

Both fresh and preserved specimens were dissected for in situ observation of internal organs. Sepioids were opened via a single median incision of the dorsal mantle wall from anterior to posterior. The mantle was then pinned aside to expose organs within the mantle cavity.

Experimental Treatments

Sepioids were maintained under one of two feeding regimens: (1) a high feeding regimen consisting of access to unlimited *A. s. australis* and (2) a low feeding regimen consisting of two to three *A. s. australis* per day. The high feeding regimen was considered representative of food levels within the natural habitat of *I. pygmaeus*, as *A. s. australis* are abundant in waters around Townsville all year (Robertson and Duke 1987; Jackson 1991; pers. obs.). The low feeding regimen was assigned after observation of a captive specimen of *I. pygmaeus* during January 1991. This individual was able to consume 8–10 *A. s. australis* during an hour-long feeding session. On this basis, two to three shrimps per day was considered an appropriate low feeding level.

Statistical Analyses

Statistical analyses were performed using the microcomputer software package Statistix 3.1, produced by the Analytical Software Corporation. Data obtained from the daily collection, drying, and weighing of eggs allowed the following aggregated

measures to be calculated for each female sepioid: (1) total number of eggs laid (Teggs), (2) total dry weight of eggs laid (Twt), (3) maximum number of eggs laid in one batch (Maxbat), (4) minimum number of eggs laid in one batch (Minbat), (5) average number of eggs laid per batch (Avbat), (6) maximum dry weight of eggs laid in one batch (Maxwt), (7) minimum dry weight of eggs laid in one batch (Minwt), (8) average dry weight of eggs laid per batch (Avwt), (9) average dry weight per egg (Avwegg) (for each batch the weight per egg was calculated; the average weight per egg is the average of this value taken over all batches laid), (10) number of batches laid (Batno), (11) Duration of laying in days (Dur) (day 1 is the day the first egg batch was laid), and (12) average time in days between batches (Timbat) (calculated by dividing the laying duration by the number of batches laid). Note that values for variables based on a total value (such as Teggs or Dur) were not calculated for females killed prematurely for histological examination. Also, the eggs of six females from early in the experiment were not weighed; thus, no weight-based measures could be calculated for these specimens.

The values obtained for each aggregated variable were compared between feeding regimens using a *t*-test. The null hypothesis for this test was that there is no difference in the mean value of the aggregated variable between feeding regimens. The significance level (α) was set at 0.05. The number of variates within each sample reflects the number of females available for the calculation based on the constraints mentioned in the previous paragraph.

To compare reproductive variability within and among female sepioids, a one-way analysis of variance (ANOVA) was performed. Egg batch dry weight was chosen as the variable for analysis, as batch weight is more indicative of changes in reproductive output than egg number. Each female sepioid (not including specimens taken for histology) formed a level of the treatment. This analysis was designed to test, at the 0.05 level of significance, the null hypothesis that there is no difference in the mean batch dry weight between female sepioids maintained on the same feeding regimen. Separate analyses were performed for each feeding regimen.

Temporal changes in the reproductive output of individual sepioids, in terms of both number and weight of eggs laid, were investigated through linear regression models. For each female which laid more than four egg batches (not including specimens taken for histology), number of eggs per batch and weight of each egg batch were regressed separately against day of laying. Day 1 was taken to be the day the first egg batch was laid. For each model, the null hypotheses H_0: slope of the fitted line = 0 was tested at the 0.05 level of significance via a *t*-test. A slope not significantly different from zero indicates that day of laying did not significantly affect the size of the reproductive output.

Results

Reproductive Behaviour in Captivity

Captive female *I. pygmaeus* attached eggs in groups to surfaces inclined 90–180° from horizontal, such as the underside of shells or rocks in the aquaria. Each egg, enveloped by a small individual gel coat, was embedded in a larger gel matrix which surrounded the whole egg batch. Eggs were typically 2 mm in diameter (including the inner gel coat). Females were consistent in their deposition sites from batch to batch, yet the favoured site usually differed between subsequent females occupying the same aquarium. We observed females laying at a rate of one egg approximately every 30 s; in other respects the egg laying behaviour was consistent with that described by Natsukari (1970). The majority of egg laying took place from 18:00 to 06:00.

Captivity did not shorten the reproductive life of *I. pygmaeus* but appeared to retard growth, as no females attained the large sizes often observed in wild females. First maturity in captive sepioids was observed at approximately 10 mm dorsal mantle length, probably equivalent to 40–60 d old (Jackson 1989). Given that the maximum age reported by Jackson (1989) was 80 d, the laying durations observed for the high-food females in this study (mean = 18 ± 2 d, max = 32 d) suggest that captivity was not shortening the reproductive life and that approximately 80 d is a realistic estimate of the life span of this species. Many specimens were maintained from an immature state until the end of their reproductive life, 45 d being the maximum observed captive duration.

No captive *I. pygmaeus* achieved the maximum size (18.5 mm dorsal mantle length, 822 mg) observed for wild-caught specimens. Mean weight at death (where this could be calculated) was 288 mg (SE = 27, n = 10) for the high-food specimens and 287 mg (SE = 21, n = 16) for low-food specimens. Large specimens (600–700 mg) taken from the wild were capable of high rates of egg production, often in excess of 50 eggs per day. Reduced growth rate, and hence maximum size, in captivity is a likely result of the stress of confinement and associated tentacular damage from rubbing against the walls of the tank while swimming.

Female *I. pygmaeus* died 6–36 h after they laid the last egg batch. Senescent sepioids typically developed skin lesions; condition deteriorated rapidly after this point, and death followed less than 24 h after the first lesion in all observed cases. Motor control was also lost in many instances, resulting in erratic and uncoordinated swimming.

Structure and Histology of the Female Reproductive System

The reproductive system of female *I. pygmaeus* consists of ovary, oviduct, oviducal gland, and paired nidamental glands. Accessory nidamental glands, often conspicuous in other cephalopod species (Mangold 1987), were not found. The oviduct is membranous and envelops the ovary, facilitating capture of mature oocytes as they are released. Posteriad the oviduct is sacular, allowing the storage of mature oocytes. At laying, oocytes pass from the oviduct through the oviducal gland which secretes a protective coating around the oocyte. Coated oocytes then pass along a duct from the oviducal gland which deposits them near the funnel opening. The nidamental glands are extremely large and occupy more than half the mantle cavity in mature female *I. pygmaeus*. The glands secrete a jelly which surrounds the oocytes as they pass out through the funnel for deposition.

Mature female *I. pygmaeus* have oocytes of many different sizes developing simultaneously, and maturation occurs continuously. Developing cephalopod oocytes are characterised by a follicular epithelium which surrounds the ova (Arnold and Williams-Arnold 1977; Knipe and Beeman 1978). Follicle cells attach to newly formed oocytes and proliferate on their surface, invaginating the cytoplasm and eventually fusing to form a syncytium. The follicular epithelium is instrumental in transporting yolk precursors into the developing oocyte and degenerates to form the chorion in mature oocytes (O'Dor and Wells 1973; Arnold and Williams-Arnold 1976). Staging schemes of varying complexity have been proposed for cephalopod oocytes, based on characteristics of the oocyte nucleus and follicular

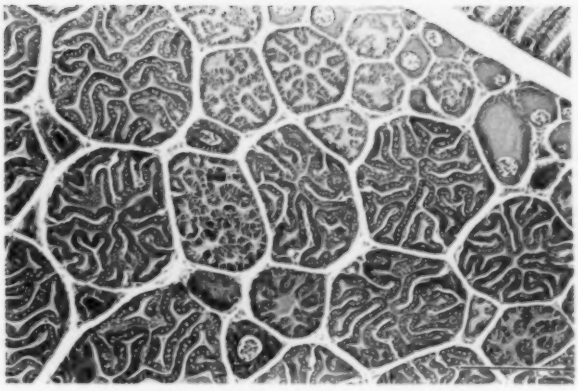

FIG. 1. Ovary of an immature *I. pygmaeus*, showing a predominance of stage 1 and 2 oocytes. Stage 1 oocytes are smaller and have distinct nuclei. Bar = 270 μm.

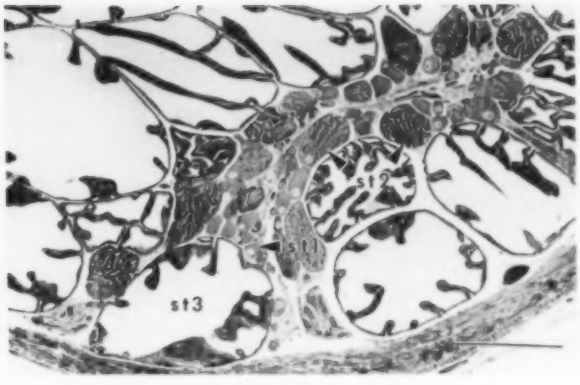

FIG. 2. Ovary of a mature *I. pygmaeus*, showing oocytes of developmental stages 1–3. st1 = stage 1 oocyte, st2 = stage 2 oocyte, st3 = stage 3 oocyte. Bar = 600 μm.

epithelium (Arnold and Williams-Arnold 1977 [after Cowden 1968]; Knipe and Beeman 1978; Coelho 1990; Sauer and Lipinski 1990).

Oocytes of *I. pygmaeus* were classified into four broad developmental stages on the basis of microscopic appearance after sectioning and staining with Mallory-Heidenhain stain.

Stage 1: small, newly formed oocytes, including primary oogonia. Oocyte nuclei are distinct. Follicular cells begin to attach to the oocyte. Formation of the follicular epithelium marks the end of this stage.

Stage 2: cells of the follicular epithelium undergo rapid division, forming convolutions which invade the oocyte cytoplasm. Oocyte nuclei remain distinct, although less frequently observed in section. Syncytium formation marks the end of this stage.

Stage 3: postsyncytium oocytes. Vitellogenesis begins and granular yolk (staining light blue) appears in the oocyte. Epithelial convolutions are strong initially but are gradually forced toward the oocyte periphery by increasing yolk volume. Epithelium convolutions stain blue/purple; follicular nuclei stain red and become indistinct at the end of this stage. Degeneration of the follicular epithelium and formation of the chorion membrane mark the end of this stage.

Stage 4: mature ova shed from the ovary, thin walled and replete with yolk. Chorion membrane surrounds the oocyte. Stage 4 oocytes are found either within the oviduct or associated with the oviducal gland.

Ovaries of immature females contained primarily stage 1 and 2 oocytes (Fig. 1), while those in mature specimens were spread over the whole continuum of developmental stages (Fig. 2). The bulk of ovary volume is taken up by large, yolk-filled stage 3 oocytes in mature sepioids, together with a variable number of mature ova (stage 4) within the oviduct, depending on elapsed time since the last spawning episode. At least two to three stage 4 oocytes were observed in all mature females, indicating that oocytes mature continuously.

Oocytes of differing developmental stages were not randomly dispersed within the ovary. Rather, oocyte size was clearly related to distance from the centre of the ovary, oocytes becoming larger toward the periphery (Fig. 2). This characteristic was confirmed via dissection. Oocytes surround the central ovarian blood vessel, oocyte primordia entally, and largest oocytes situated ectally, giving the ovary a botryoidal appearance.

Histological Appearance of Senescent Sepioids

Senescence in *I. pygmaeus* occurred rapidly and independently of the exhaustion of reproductive potential. Skin lesions, which typically developed on the head or mantle, indicate the terminal stage of senescence, as death followed less than 24 h after their appearance in all cases. Many specimens in the terminal stages of senescence were preserved and sectioned; their internal characteristics were consistent between individuals. In all cases the digestive caecum had broken down, the internal structure becoming sparse and fragmented, and in some specimens it was completely absent. The appearance of the caecum was distinctly different from that found in healthy specimens which had been deprived of food, where the organ appeared normal but greatly reduced in size. This suggests that senescent specimens were slowly starving due to loss of digestive function. Consistent with these observations, senescent specimens declined food even though their tentacular apparatus appeared in working order. Several senescent males that were examined microscopically showed a breakdown of the caecum similar in all respects to that of the female specimens.

Ovaries of senescent specimens still contained a large number of immature oocytes of stages 1 and 2 and submature oocytes of stage 3. Stage 4 oocytes were absent in most cases, or in very low numbers. Senescence in *I. pygmaeus* appears to be initiated by digestive failure, effectively halting the process of oocyte maturation. The absence of stage 4 oocytes in senescent specimens suggests that the dying female is capable of laying those oocytes contained in the oviduct at the onset of senescence. Development of skin lesions, possibly linked to failure of the immune system, occurs after the final egg laying and would appear to be the ultimate cause of mortality.

Reproductive Characteristics of *I. pygmaeus*

In captivity, *I. pygmaeus* produced multiple egg batches. Features of the reproductive output such as batch size and time between batches were consistent within individual sepioids, yet varied between individuals.

The aggregated variables (Table 1) show that, with unlimited access to food, female *I. pygmaeus* produce on average 65 eggs every 2 d over an 18-d period, resulting in a total egg output of around 650 eggs. Remarkably, the maximum observed output was more than double these averages. Two sepioids laid

TABLE 1. Means for aggregated measure variables (defined in the text) characterising the reproductive output of *I. pygmaeus*. The coefficient of variation (CV) for each variable appears in parentheses. The probabilities of means being similar between food groups, as calculated via a *t*-test, are also shown. Weights are dry and in milligrams; times are in days.

	High food			Low food			
Variable	Mean	(CV)	*n*	Mean	(CV)	*n*	*P*
Teggs	641	(0.57)	14	322	(0.71)	22	0.0028
Twt	1028	(0.62)	12	530	(0.75)	20	0.0104
Maxbat	105	(0.38)	23	73	(0.57)	27	0.0089
Minbat	42	(0.72)	23	21	(0.64)	27	0.0021
Avbat	65.2	(0.43)	23	41.4	(0.50)	27	0.0010
Maxwt	154	(0.38)	19	113	(0.43)	24	0.0179
Minwt	57	(0.53)	19	31	(0.63)	24	0.0023
Avwt	98	(0.36)	19	66	(0.67)	24	0.0050
Avwegg	1.72	(0.20)	19	1.66	(0.18)	25	0.5647
Batno	11.2	(0.43)	14	7.6	(0.62)	22	0.0345
Dur	18	(0.41)	14	14.7	(0.51)	22	0.2070
Timbat[a]	1.60	(0.16)	14	2.04	(0.28)	22	0.0080
Timbat	1.81	(0.37)	23	2.10	(0.25)	27	0.1000

[a]Refers to the *t*-test for the variable Timbat calculated on data from the same specimens used to calculate the *t*-tests for Dur and Batno.

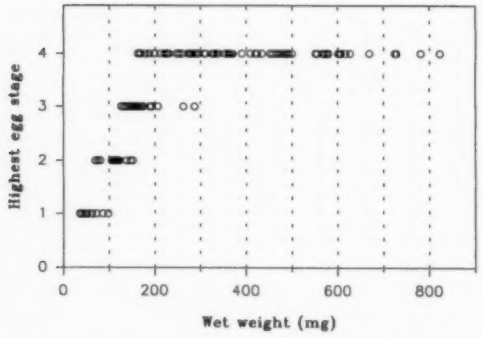

FIG. 3. Relationship between highest microscopically observable egg stage and female wet weight of *I. pygmaeus*.

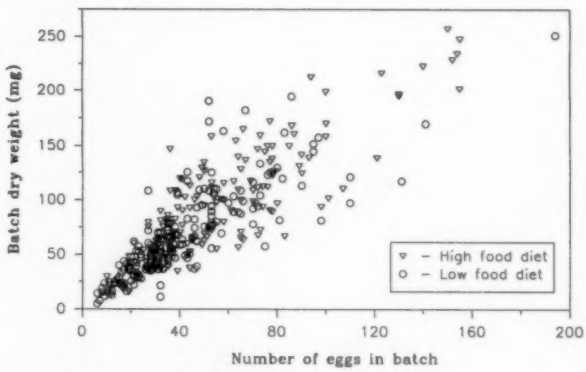

FIG. 4. Relationship between dry weight and number of eggs for egg batches ($n = 338$) collected from female *I. pygmaeus* of both feeding regimens.

more than 1150 eggs, while one specimen laid 1215 eggs in 12 batches over the course of 25 d. The greatest number of egg batches laid by a completely satiated sepioid was 19. There is no significant correlation between rate of egg production and duration of laying (linear regression, $p = 0.318$); overall, the most fecund sepioids laid a greater number of eggs simply because they survived for longer than the less fecund sepioids. Average time between batches was slightly less than 2 d. The majority of specimens laid eggs every second day, with occasional batches on consecutive days.

Reproductive output varied considerably between individuals. The coefficients of variation (Table 1) indicate high variability about the mean for all aggregated measures, with the exception of *average weight per egg*. Mean batch weight was different between females (one-way ANOVA, $P < 0.0001$), indicating that interindividual variations in reproductive output are greater than intraindividual variations.

To gauge size-specific levels of oocyte maturity, 110 predominantly wild-caught female *I. pygmaeus* were sectioned and examined as described previously. The most mature oocyte stage observable in section plotted against wet weight (Fig. 3) allows some generalisations to be made regarding maturity in *I. pygmaeus*. Females of this species are sexually mature over most of the observed size range found in the wild (150–850 mg). However, due to the exponential growth rate of the animal (derived from Jackson's 1989 data), maturity occurs over a proportionally smaller range of ages (days 40–80).

Female *I. pygmaeus* appear to become mature at 150–200 mg and 9–11 mm, which is smaller than Jackson's (1989) estimate of 400 mg and 13 mm. There is considerable overlap between weight and oocyte stage, indicating interindividual variation in reproductive maturity of wild specimens. Final maturity occurs quite rapidly; ovaries progress from stage 1 to stage 4 in conjunction with a 100-mg change in body weight.

Effect of Feeding Regime on Reproductive Output

Female *I. pygmaeus* in the low-food treatment generally produced smaller batches of normal-sized eggs with the same frequency as those fed optimally (Table 1). The variables *average weight per egg* and *time between batches* are not significantly different between regimens, while *average batch size* is significantly reduced in the low-food sepioids. Importantly, egg quality (as far as can be discerned from values of dry weight per egg) does not appear to be affected.

TABLE 2. Results of fitting linear regression models to plots of number of eggs per batch, and batch dry weight against day of laying, for 34 female *I. pygmaeus*. *P* refers to the probability that the slope of the fitted line equals zero (*t*-test at the 0.05 level of significance). Where the slope is significantly different from zero, the sign in parentheses indicates whether the coefficient is positive or negative. *n* refers to the number of egg batches laid by a given female.

High-food			Low-food		
P egg number	*P* batch weight	*n*	*P* egg number	*P* batch weight	*n*
0.444	0.360	16	0.071	0.507	9
0.943	0.399	5	0.815	0.438	6
0.587	0.082	9	0.023 (−)	0.125	20
0.139	n/a	12	0.004 (−)	0.098	6
0.074	0.025 (−)	13	0.410	0.373	13
0.004 (−)	0.004 (−)	12	0.333	0.086	6
0.489	0.076	8	0.882	0.829	6
0.924	0.144	19	0.274	0.283	8
0.007 (−)	0.095	15	0.089	0.592	5
0.594	0.257	8	0.264	0.593	5
0.025 (−)	0.815	5	0.005 (−)	0.005 (−)	6
0.430	0.554	17	0.548	0.466	8
0.395	0.513	15	0.339	0.900	5
0.312	0.850	13	0.084	0.119	9
0.899	0.901	13	0.161	0.278	6
0.063	n/a	16	0.877	n/a	6
0.379	0.622	7	0.219	0.914	19

Variation in reproductive output previously noted for the high-food sepioids was also manifest in the low-food animals, together with the trend of lowest variation amongst *average weight per egg*. Low-food specimens differed in mean batch weight between females (one-way ANOVA, $P < 0.0001$). In general, considerable overlap in the range of values for each variable occurred between food regimens. For example, three specimens from the low feeding regimen produced in excess of 600 eggs, while the longest laying duration of any sepioid (35 d) was recorded from a female on this regimen.

Although there were large interindividual variations in reproductive output, weight per egg was similar between food groups (Fig. 4). After log transforming dry weight and number of eggs in a batch ($x' = \ln(x)$) to ensure constant error variance, an *F*-ratio test revealed that a multiple regression model consisting of fitted lines with different slopes for each food group did not explain the data significantly better than a model consisting of two lines with equal slopes for each food group [multiple regression $F(1,335) = 2.365$, $p = 0.12503$]. As the slopes represent weight per egg, this regression analysis reinforces the result obtained from the *t*-test which showed no difference in *average weight per egg* between food groups. High variation in dry weight is evident in egg batches from both feeding regimes, especially for those containing more than 40 eggs.

Histological differences were noted in the ovaries of female sepioids according to feeding history. In females maintained under the low-food regimen, the ovary occupied a smaller volume within the body cavity than in the high-food specimens. The majority of oocytes in the ovary were of stages 1, 2, and early 3; late stage 3 and stage 4 oocytes were uncommon, especially in sepioids which had recently spawned. In contrast, high-food females which had recently spawned usually had large numbers of stage 3 oocytes within their ovaries. Otherwise, sepioids from both regimes were smaller with regard to microscopic structure and condition of organs. Of particular note is the fact that in all wild females sectioned, the state of the ovary was analogous to that found in the high-food specimens.

Temporal Changes in Reproductive Output

Captive *I. pygmaeus* generally produced egg batches of consistent size and weight over the course of their reproductive life. A small percentage of specimens showed a decline in batch size through time, although this trend was not necessarily related to a decline in batch weight. Increases in batch size or mass through time were rare.

Temporal changes in reproductive output (number of eggs per batch and batch weight) were investigated for 34 female sepioids, equally split between food regimens. Linear regression models were fitted to the plots of batch size and batch weight for each individual (Table 2). Six individuals, three from each regimen, showed a decline in batch size through time, indicated by negative slopes significantly different from zero. All other specimens showed no significant change in number of eggs per batch through time. Of the six sepioids which showed declines in number of eggs per batch through time, only two (one from each food regimen) showed simultaneous declines in batch weight. All other specimens showed no significant change in batch weight through time.

Organic Content of *I. pygmaeus* Eggs

Female *I. pygmaeus* incorporated large amounts of organic material into gametes during a reproductive life of less than 30 d. Most specimens from the high feeding regimen produced eggs weighing at least twice their body weight (in terms of dry weight), while one sepioid produced more than nine times its body weight over 25 d.

Initially batch organic weight was plotted against batch dry weight. Fitting a linear regression model to the data revealed a nonnormal distribution of error variance; therefore, a natural logarithmic transformation ($x' = \ln(x)$) was applied to both variables. Replotting the log-transformed data revealed a linear relationship with constant error (Fig. 5; Table 3).

For the dry egg batches from each female *I. pygmaeus*, this regression model was used to estimate organic weight of each batch. By addition, an estimate of the total organic material

TABLE 3. Results of the linear regression of ln(batch organic weight) against ln(batch dry weight) for egg batches produced by captive female *I. pygmaeus*.

Predictor variables	Coefficient	Standard error	Student's *t*	*P*
Intercept	−0.705	0.099	−7.10	<0.0001
Slope	0.934	0.029	40.69	<0.0001
Degrees of freedom:	69			
Adjusted R^2	0.9594			
Residual mean square	0.01109			

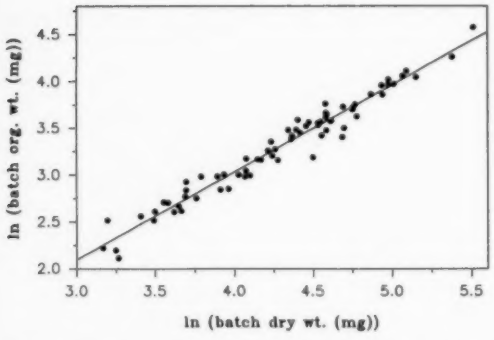

FIG. 5. Relationship between egg batch organic weight, obtained from the ashing process, and egg batch dry weight. Line of best fit is shown. Data have been transformed (natural log) to ensure constant error variance.

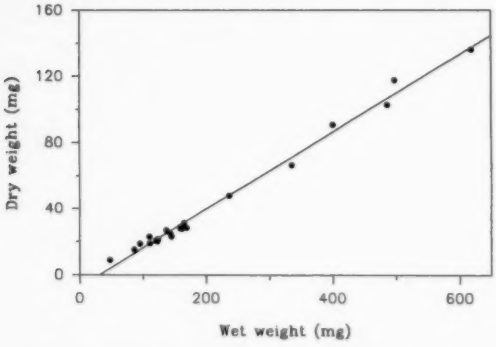

FIG. 6. Relationship between freeze-dried weight and wet weight for a range of specimens of *I. pygmaeus*. Line of best fit is shown.

incorporated into eggs, together with an associated error, could be made for each female.

To express the dry organic reproductive output as a ratio of female dry body weight, a relationship was needed to estimate female dry weight from the known wet weight at death. Dry weight was regressed against wet weight for the 22 freeze-dried specimens (Fig. 6) and the model (Table 4) used to estimate dry body weight and associated error for all females with a reliable dead wet weight (no significant decomposition).

The estimated organic reproductive output in terms of dry female body weight at death, together with an associated error, was calculated for all female *I. pygmaeus* with a reliable dead wet weight (Table 5). It should be noted that female weight at death is likely to be slightly lower than the healthy "laying" weight, due to the effects of senescence. However, based on the speed of senescence, this difference is estimated to be less than 5% in most cases.

These data reinforce the notion that *I. pygmaeus* has an extremely high commitment to reproductive output, as suggested by the aggregated variables describing fecundity. On average, specimens maintained on the high-food diet incorporated 5.1 times their body weight into reproductive material over a mean laying duration of 18 d; all specimens laid their eggs in under 32 d. The specimen with the maximum total egg number also produced the highest organic output, around nine times her body weight in 25 d. Specimens maintained on the low-food diet produced lower amounts of organic material, averaging 2.8 times their body weight over a mean laying duration of about 15 d. The most fecund specimen from this regime produced 6.5 times her body weight over 35 d.

Discussion

This represents the first comprehensive study of reproductive effort and its allocation through time in a tropical cephalopod. The age- and size-specific reproductive schedules of *I. pygmaeus* now form references against which demographic traits of other cephalopods can be examined. The usefulness of *I. pygmaeus* as a reference is through the correlation of its anatomical features with known aspects of the reproductive output, in particular those relating to multispawning. For instance, Harman et al. (1989) suggested that continuous oocyte maturation and partially filled oviducts indicate a multispawning capability for *S. oualaniensis*, yet no direct evidence was available. In *I. pygmaeus*, such anatomical features can be directly related to repeated spawning, supporting the suggestions of Harman et al. (1989).

O'Dor and Webber (1986) suggested that semelparity and associated simplistic hormonal controls over reproduction may be widespread amongst coleoid cephalopods. While we do not attempt to review the incidence of terminal spawning within cephalopods, the study does reconfirm evidence (Rodaniche 1984; Harman et al. 1989) indicating that multispawning and associated complex regulatory mechanisms (presumably hormonal) are present within the Cephalopoda.

Idiosepius pygmaeus is capable of producing multiple egg batches consistently spaced over a wide adult size range, confirming the initial expectations of Jackson (1991) who found multiple spawning in a smaller sample of specimens. The reproductive output is produced in the latter third or quarter of the life span. In these respects, *I. pygmaeus* is a true multiple spawner, as defined by Harman et al. (1989) and Kirkendall and Stenseth (1985), rather than an intermittent semelparous spawner where the products of a single bout of oocyte maturation are released at several intervals through time. This species is functionally iteroparous from both an endocrine and physiological perspective. The presence of stage 1 oocytes within

TABLE 4. Results of the linear regression of dry body weight against wet body weight for *I. pygmaeus*.

Predictor variables	Coefficient	Standard error	Student's *t*	*P*
Intercept	−7.193	1.283	−5.61	<0.0001
Slope	0.234	0.005	47.28	<0.0001
Degrees of freedom:	20			
Adjusted R^2	0.9907			
Residual mean square	12.32			

TABLE 5. Estimates of total dry weight of organic material incorporated into eggs by *I. pygmaeus*, expressed as a ratio of the dry female body weight at death. All weights in milligrams; standard errors in parentheses.

Food level	Female dead weight	Estimated female dry weight (*F*)	Total egg dry weight	Estimated egg organic weight (*E*)	Ratio *E*:*F*
High	291.0	61 (0.8)	1552.1	472 (87)	7.7 (1.4)
High	280.0	58 (0.8)	386.3	129 (6)	2.2 (0.1)
High	306.0	64.5 (0.9)	1955.5	585 (77)	9.1 (1.2)
High	482.3	106 (1.5)	823.1	261 (21)	2.5 (0.2)
High	340.0	72 (0.1)	1333.5	506.7[a]	7.04 (0.01)
High	175.5	34 (0.8)	207.9	72 (2)	2.12 (1.08)
High	168.0	32 (0.8)	363.8	122 (8)	3.8 (0.3)
High	272.7	57 (0.8)	376.9	126 (5)	2.21 (0.09)
High	294.0	62 (0.9)	1817.6	547 (110)	8.8 (1.8)
High	272.5	57 (0.8)	996.0	312 (44)	5.5 (0.8)
Low	247.2	51 (0.8)	291.4	99 (7)	1.94 (0.14)
Low	180.1	35 (0.8)	216.7	75 (2)	2.14 (0.07)
Low	356.0	76 (1)	1393.8	427 (97)	5.6 (1.3)
Low	198.2	39 (0.7)	235.2	81 (3)	2.08 (0.08)
Low	173.7	33.5 (0.8)	70.9	26 (0.02)	0.78 (0.02)
Low	232.7	47 (0.8)	272.4	93 (4)	1.98 (0.09)
Low	278.5	58 (0.8)	526.6	172 (6)	2.96 (0.1)
Low	261.0	54 (0.8)	469.1	154 (11)	2.9 (0.2)
Low	353.2	75.6 (1)	178.7	63 (2)	0.83 (0.02)
Low	252.8	52 (0.8)	382.4	128 (4)	2.46 (0.09)
Low	409.0	89 (1.2)	1322.3	497.2[a]	5.58 (0.08)
Low	264.0	55 (0.8)	241.9	83 (3)	1.51 (0.06)
Low	334.2	71 (1)	676.3	217 (17)	3 (0.2)
Low	492.0	108 (1.6)	860.6	272 (14)	2.5 (0.1)
Low	276.0	57.5 (0.8)	355.2	119 (6)	2.1 (0.1)
Low	287.0	60 (0.8)	1259.0	388 (82)	6.5 (1.4)

[a]Indicates an actual value derived from the ashing process.

all specimens, even those which had senesced, further reinforces the notion of multiple spawning and continuous oocyte maturation. Terminally spawning cephalopods may show a continuum of oocyte stages at small sizes, but all egg stages have matured as spawning approaches (Arnold and Williams-Arnold 1977; Coelho 1990; Sauer and Lipinski 1990).

The results show *I. pygmaeus* to be capable of detailed regulation of reproductive output. The small size at maturity of *I. pygmaeus* relative to the maximum attainable size means that massive reproductive output (Table 5) and growth are coordinated simultaneously. With the exception of *S. oualaniensis* (Harman et al. 1989) and *O. chierchiae* (Rodaniche 1984), most studies of reproduction and growth in cephalopods have suggested that egg laying and growth are mutually exclusive, with the onset of laying signalling a decline or even complete termination of growth (Bergstrom and Summers 1983; Hanlon 1983a, 1983b; O'Dor 1983; Okutani 1983; Worms 1983; Boletzky 1987; Forsythe and Van Heukelem 1987; Boyle 1990). *Idiosepius pygmaeus* is clearly an exception to this trend.

A wide body of theoretical literature exists regarding trade-offs between reproduction and female survival. A female organism under food stress may maintain reproductive output at the expense of somatic maintenance and hence longevity (Stearns 1989; Kaitala 1991) or reduce reproductive output, thereby maintaining or even lengthening life span (Spight and Elmen 1976; Ernsting and Isaaks 1991; Qian and Chia 1991). *Idiosepius pygmaeus* appears to adopt the latter strategy which intrinsically requires more flexibility in the digestive, metabolic, and reproductive systems. The ability in *I. pygmaeus* to maintain egg size under food stress (Table 1; Fig. 4) must involve either an increase in oocyte developmental time or an active partitioning of yolk precursors between oocytes within the ovary or (more likely) a combination of both.

Periodicity of egg laying was maintained between food groups, even though the mean number of eggs laid on each occasion was lower in females from the low-food regime (Table 1). This result indicates that a full oviduct cannot be the spawning stimulus; if it were, females from the low regime

would be expected to show a longer interbatch period yet lay egg batches of size similar to those of high-food females. An internal mechanism must therefore exist to control timing of egg laying independent of oviduct fullness.

These three factors, coordination of reproduction and growth, regulation of egg size, and control over timing of laying, in conjunction with characteristics such as internal fertilisation, sperm storage, large somatic commitment to reproductive glands, and direct development of hatchlings all reveal that cephalopods can indeed develop a reproductive system of complexity comparable with their advanced nervous, circulatory, and digestive systems. The endocrine system of *I. pygmaeus* is the likely candidate for control of such processes, given the central role of this system in maturation and reproduction of octopods (Arnold and Williams-Arnold 1977; Boyle and Thorpe 1984; O'Dor and Webber 1986) and other molluscs in general (Arnold 1984). However, current endocrine models (based on octopods) are too simplistic to explain multiple spawning (Mangold 1987), suggesting that a reassessment of endocrinology based on a multispawner such as *I. pygmaeus* is needed to reveal the true complexity of this system within the Cephalopoda. These results confirm O'Dor and Webbers' (1986) suggestion that cephalopods should not be prevented by their molluscan heritage from possessing advanced reproductive systems, and provide new evidence that semelparity and endocrine simplicity cannot be assumed within the Cephalopoda.

Acknowledgements

Leigh Winsor and Zolly Florian provided assistance with histology and photography, respectively. We thank Natalie Moltschaniwskyj and George Jackson for discussions during this study and for reading the manuscript. This study was supported partly by an ARC Minor grant to J.H.C. and by internal funds from the James Cook University Research Panel.

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Complex Interactions of Multiple Aquatic Consumers: An Experimental Mesocosm Manipulation

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Richardson, W. B., and S. T. Threlkeld. 1993. Complex interactions of multiple aquatic consumers: an experimental mesocosm manipulation. Can. J. Fish. Aquat. Sci. 50: 29–42.

In 7-m^3 outdoor tanks filled with lake water, the presence/absence of omnivorous young-of-the-year *Micropterus salmoides*, zooplanktivorous *Menidia beryllina*, and herbivorous larval *Hyla chrysocelis* was experimentally manipulated. A cross-classified design was used to assess the interactive effects of these vertebrate consumers on the experimental food webs. Large zooplankters and large, actively swimming macroinvertebrates (*Ceriodaphnia*, *Simocephalus*, Corixidae, Notonectidae, and Dytiscidae) were particularly susceptible to depletion by both fish species. *Micropterus* tanks contained greater numbers of *Keratella quadrata* and Hebridae but fewer benthic cyclopoid copepods. *Menidia* tanks contained more *Synedra* and algal filaments. *Hyla* exerted only minor direct effects on the tank communities. When both fish species were in the same tank, both had elevated mortality. *Micropterus*, in tanks with *Menidia* and *Hyla*, were larger and in better condition than in tanks without *Hyla*. The primary effects of the experimental manipulations on food web components were two- and three-way interactions in which the effect of a given treatment was dependent on the presence of another treatment. Results suggest that the addition or removal of consumers may not cause linear, additive changes in food webs.

Dans des bassins extérieurs de 7 m^3 remplis d'eau de lac, on a expérimenté des combinaisons de jeunes de l'année omnivores de *Micropterus salmoides*, de *Menidia beryllina* zooplanctivore et de larves herbivores de *Hyla chrysocelis*. On a évalué par classement croisé les effets de caractère interactif que ces vertébrés consommateurs exercent sur des pyramides alimentaires expérimentales. Les gros zooplanctivores et les gros macroinvertébrés nectoniques (*Ceriodaphnia*, *Simocephalus*, Corixidae, Notonectidae et Dytiscidae) étaient particulièrement sensibles à une déplétion par les poissons des deux espèces. Les bassins de *Micropterus* contenaient des *Keratella quadrata* et des Hebridae en plus grand nombre, mais moins de copépodes cyclopoïdes benthiques. Les bassins de *Menidia* contenaient davantage de *Synedra* et d'algues filamenteuses. *Hyla* n'a eu d'effets directs que mineurs sur les communautés des bassins. Lorsque les poissons des deux espèces étaient introduits dans un même bassin, les deux souffraient d'une mortalité élevée. Dans les bassins de *Menidia* et d'*Hyla*, les *Micropterus* étaient plus gros et en meilleure condition que ceux des bassins sans *Hyla*. Les effets primaires des expérimentations sur les composants de la pyramide alimentaire étaient des interactions à deux et trois sens dont on peut dire que l'effet d'un traitement donné était dépendant de l'application d'un autre traitement. Les résultats donnent à penser que l'addition ou la suppression de consommateurs peut ne pas provoquer de changements additifs et linéaires dans les pyramides alimentaires.

Received April 9, 1991
Accepted August 5, 1992
(JA986)

Reçu le 9 avril 1991
Accepté le 5 août 1992

The process by which we have gained an understanding of the impact of consumers on aquatic ecosystems includes manipulation of individual consumers (e.g. Ball and Hayne 1952; Hrbáček 1962; Hillbricht-Ilkowska 1964; Brooks and Dodson 1965; Keast and Webb 1966; Macan 1966; Novotna and Korinek 1966; Macan 1977; Northcote 1988). Typically, these workers have studied either accidental introductions or experimental manipulations of a single predator on some portion of the food web (but see Hall et al. 1970). Extrapolation beyond these studies to community-level effects assumes that species in a community interact as building blocks: when single species are placed with other species, they function as they do alone (i.e. simple additive effects). This assumption leaves unresolved the possible complex relationships and effects of multiple life stages, consumer types, and species interactions that are probably the norm of ecosystem functioning (Abrams 1983; Pimm and Rice 1987; Morin et al. 1988; Richardson et al. 1990; Wilbur and Fauth 1990).

The objective of our experiment was to test the hypothesis that multiple consumer types (zooplanktivory, omnivory, and periphyton grazing) would have direct as well as interactive effects on the entire suite of prey in large experimental tanks. Significant statistical interaction effects would lead us to reject the hypothesis that an effect of a particular consumer was independent of the presence of another consumer ("building-block hypothesis"). In a replicated, cross-classified experiment, we manipulated the presence/absence of three consumers (*Micropterus salmoides*, *Menidia beryllina*, and *Hyla chrysoscelis* tadpoles). This design allowed us to explore the main effect of each consumer and the interactive effects between the consumers and the entire tank community. We chose these particular organisms for manipulation because their feeding would likely affect standing stocks of the major components of the tank food webs: zooplankton by *Menidia* (e.g. Saunders 1959), periphyton by tadpoles (e.g. Dickman 1968; Seale 1980), and both planktonic and benthic invertebrates by *Micropterus* (e.g.

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Keast et al. 1978; George and Hadley 1979; Hamilton and Prowles 1983). We suspected that the impact of the feeding activity of the two fish would be additive, i.e. the presence of both fish in the same tank would compound the effect of any one fish treatment alone. The tadpoles were expected to reduce periphyton mass and, indirectly, enhance phytoplankton density via nutrient release (Dickman 1968; Seale 1980; Osborne and McLachlan 1985).

Materials and Methods

The experiment was conducted in 18 insulated fiberglass tanks located outdoors at the University of Oklahoma Biological Station, Kingston, OK. Tanks were 2 m high, 2.3 m in diameter (4.3 m^2), and contained 7000 L of water pumped from Lake Texoma and filtered through a 63-μm-mesh net. An airlift mixing system prevented thermal stratification, moving approximately 85 L water·min^{-1} from 50 cm above the bottom to the surface of the tanks. Tanks were sampled from an elevated walkway. These units reduced the problems of inaccessibility to sampling and notoriously high within-site variability of benthic communities (Needham and Usinger 1956; Resh 1979; Allan 1984; Resh and Rosenberg 1989). Variability in benthic communities has hindered the ability to detect effects of food web manipulations. Identical experimental tanks reduce some major sources of variability associated with differences in the physical attributes of habitat, particularly substrate, cover, temperature, and depth (Downing 1979; Mittelbach 1981; Harper and Cloutier 1986).

The experiment was a cross-classified design. Treatment combinations included two tanks each of the following: control (no vertebrates), *Menidia* alone, *Micropterus* alone, *Hyla* alone, *Menidia* + *Micropterus*, *Menidia* + *Hyla*, *Micropterus* + *Hyla*, and *Menidia* + *Micropterus* + *Hyla*. Treatments were assigned to tanks at random within this design. Tanks were filled 5 May 1987 and approximately 500 mL of Lake Texoma zooplankton was added. Sampling began 2 June, immediately after addition of fish and tadpoles, and ended 28 June. The period between tank filling and the beginning of the experiment allowed colonization of the tanks by insects and development of plankton populations. *Menidia beryllina* (Cope) (Atherinidae) were seined from Lake Texoma, held for 48 h, and released, 20·*Menidia* $tank^{-1}$ (0.32 g·m^{-2}). Young-of-the-year *M. salmoides* (Lacepede) (Centrarchidae) were acquired from the State Fish Hatchery at Holdenville, OK, and held for 72 h before stocking at 10·*Micropterus* $tank^{-1}$ (0.43 g·m^{-2}). Larval *H. chrysoscelis* (Cope) (Amphibia: Hylidae) were transferred from tubs (in which natural oviposition had occurred) to clean tubs for 24 h and then into the experimental tanks (135 tadpoles·$tank^{-1}$, 0.32 g·m^{-2}). Food was withheld from fish and tadpoles during the holding period to reduce introduction of nutrient-containing gut contents. Standard and total lengths of fish, body lengths of tadpoles, and dry weights of fish and tadpoles were measured on subsampled individuals for estimation of length–weight relationships to be used in the assessment of mass changes.

The tanks were sampled at 5-d intervals. Duplicate zooplankton samples were taken by vertical tows of a Wisconsin net (80-μm mesh) and subsequently preserved in 4% sucrose–formalin. Subsamples (10–20% volume) were counted at 20–40× using a Wild dissecting microscope. Identification of zooplankton followed Edmondson (1959), Pennak (1978), Smith and Fernando (1978), and Stemberger (1979). Odonate emergence and aborted emergence attempts (based on remaining exuvia) were recorded daily.

Whole-water samples were taken from the airlift ports for measurement of several variables. In vivo algal chlorophyll fluorescence and DCMU-enhanced fluorescence (DCMU = 3(3,4-dichlorphenyl)-1,1-dimethylurea) were measured using an Aminco fluorimeter. DCMU is a photosynthetic inhibitor which blocks electron transport between photosystems I and II (Vincent 1981). In doing so, energy is released in the form of fluorescence. The relative increase in chlorophyll fluorescence with DCMU, compared with without, is directly proportional to photosynthetic rates under saturating irradiance (photosynthetic capacity) and is a relative measure of health of algal cells (Samuelsson et al. 1978; Vincent 1981; Keller 1987). Suspended particle size (equivalent circular diameter, longest dimension, and particle density) was measured using a Bausch and Lomb Omnicon 5000 image analysis system. Turbidity was measured with a Hach turbidometer (model 2100A). Temperature, conductivity, dissolved oxygen, and pH were measured in situ with a Hydrolab 4041 water quality probe. Secchi depth was also measured.

At the end of the experiment (28 June 1987) the tanks were drained and fish recovered. Fish were measured (standard and total length) and then dried at 60°C for 24 h and weighed. Condition factor (k = dry weight·$length^{-3}$ × 1000) was calculated for each individual. Periphyton was harvested from the walls of each tank by scraping 10 random patches (32 cm^2 each). The scrapings were dry weighed (as above) and then ashed (550°C for 24 h) for calculation of ash-free dry weights.

After the tanks were drained, the entire contents remaining on the bottom were removed. Estimates of benthic organic matter were derived from subsampled portions of the harvest of the entire tank bottom. All material was sieved through a large screen (2.5 × 5 cm mesh) for collection of very large particles (e.g. leaf litter and filamentous algae). Benthic biomass (primarily fine organic matter, leaf fragments, and small algal filaments) and invertebrate density were taken from the remaining benthic material. The invertebrate subsample was sieved onto a No. 30 mesh screen (retains particles >0.59–0.71 mm in diameter) before preservation in formalin and rose bengal. Invertebrates were removed from 30–50% of the sample and identified to the lowest possible taxa using Needham and Westfall (1955), Wiggins (1977), Brigham et al. (1982), and Merritt and Cummins (1984).

The primary objective of this experiment was to test for the existence of interactive effects of the combinations of species. Our multilevel, factorial experimental design allowed for a test of the null hypothesis of no significant interaction effects. An interaction effect is a measure of the extent to which a response from treatment combination XY cannot be predicted from the sum of the mean responses of treatment X_i and treatment Y_j alone (main effects) (Winer 1971). Three-way analysis of variance (ANOVA) (general linear model, SAS Institute, Inc. 1990) was used as an appropriate test of response variable main effects and interactions, given the experimental design and hypothesis of interest (Winer 1971). The model used in the analysis was

$$Y_{ijk} = \mu + \beta_i + M_j + T_k + BM_{ij} + MT_{jk} + BT_{ijk} + BMT_{ijk} + e_{ijk}$$

where Y = mean density response, μ is the parametric mean of density (background effects), B_i, M_j, and T_k are the adjustments for main effects of *Micropterus*, *Menidia*, and *Hyla* (where i, j, k = 1 or 0: presence or absence), BM_{ij}, MT_{jk}, and

TABLE 1. ANOVA tables showing breakdown of class variables and assignment of degrees of freedom used to analyse variables from a manipulation of the presence/absence of *Micropterus*, *Menidia*, and *Hyla* tadpoles. (a) Three-way ANOVA table for analysis of food web variables; (b) two-way ANOVA table for analysis of fish recovery data (e.g. mass and numbers).

Source	Degrees of freedom
(a) Three-way ANOVA	
Model	7
Error	10
Total	17
Micropterus	1
Menidia	1
Hyla	1
Micropterus × *Menidia*	1
Micropterus × *Hyla*	1
Menidia × *Hyla*	1
Micropterus × *Menidia* × *Hyla*	1
(b) Two-way ANOVA	
Model	3
Error	4
Total	7
Fish (*Micropterus* or *Menidia*)	1
Hyla tadpoles	1
Fish × tadpoles	1

BT_{ik} are the adjustments for the two-way interaction effects, BMT_{ijk} is the adjustment for three-way interactions, and e_{ijk} is the remaining or within-tank variation. The ANOVA model and degrees of freedom are laid out in Table 1a.

Responses for each tank were averaged over time, after time effects were examined using repeated measures ANOVA with multivariate ANOVA (general linear model, SAS Institute, Inc. 1990). All variables were found to have insignificant time-associated variance components except *Ceriodaphnia*, *Keratella quadrata*, copepod nauplii, and 16–32 μm seston particles. Further examination of these variables showed that temporal changes in variance occurred primarily between the first and second sampling date. We feel that the lack of temporal variation justified the consolidation of time-associated variance into single tank means for further analysis. We used two-way ANOVA (fish: *Micropterus* or *Menidia* × tadpole; Table 1b.) to test for differences in recovered fish length, weight, and condition between treatments (general linear model, SAS Institute, Inc. 1990).

To assess, density-associated treatment effects, we used analysis of covariance (ANCOVA) with starting and ending fish mass and density as covariates. Here, we were attempting to evaluate the impact of additional fish bodies in tanks with multiple species of fish assigned to them, testing the null hypothesis that there was no density-dependent response of any of the variables.

Graphical presentation of the data is by way of three-way interaction plots (Winer 1971). These allow rapid evaluation of multiway interaction surfaces not discernable from either time series or bar plots of all eight treatment combinations. The location of each treatment is labeled in Fig. 1A. There are eight points on each plot, representing the least-square mean of each of the eight treatment combinations. Treatments without tadpoles are represented by the response surface bounded by the open circles and broken lines. Treatments with tadpoles are represented by the surface bounded by solid circles and solid lines. The effect size of a given treatment is measured relative to the control tank least-square mean (P. Dixon, Savannah River Ecology Laboratory, pers. comm.). Variance is indicated by a single vertical bar and is the standard error of the least-square mean estimate of treatment effects. The single estimate of variance is appropriate for this analysis, as it represents the criterion for establishing treatment difference over the entire ANOVA model (Winer 1971).

Results

Consumer Main Effects

The presence of *Micropterus* alone had few impacts on tank communities. Numbers of *Ceriodaphnia* (Cladocera), the largest phytoplankton grazer, were reduced ($P = 0.009$, Fig. 1A), while those of *K. quadrata* (Rotifera) were elevated in *Micropterus* tanks ($P = 0.034$, Fig. 1B). In the benthos, *Pantala* (Odonata) larvae ($P = 0.044$, Fig. 1C), Dytiscidae (Coleoptera) adults ($P = 0.002$, Fig. 1D) and larvae ($P = 0.002$, Fig. 1E), *Simocephalus* (Cladocera) ($P = 0.068$, Fig. 1F), and benthic cyclopoid copepods ($P = 0.043$, Fig. 1G) were less abundant in the presence of *Micropterus* than in control tanks. *Caenis* (Ephemeroptera) ($P = 0.089$, Fig. 1H) and *Lipogomphus* (Hebridae: Hemiptera) ($P = 0.039$, Fig. 1I) were more abundant in the presence of *Micropterus*.

Tanks with *Menidia* contained an extremely depauparate set of microcrustacean zooplankton taxa. *Bosmina* (Cladocera, $P = 0.0002$, Fig. 2A), *Ceriodaphnia* ($P = 0.013$, Fig. 1A), and *Diaptomus* (Calanoida) ($P = 0.02$, Fig. 2B) were reduced nearly to extinction. *Keratella cochlearis* ($P = 0.057$, Fig. 2C) was found in greatest abundance in *Menidia* tanks. The size of sestonic particles (equivalent circular diameter, $P = 0.004$, Fig. 2D) was reduced in the presence of *Menidia*. The number of particles in the size range of 8–16 μm was most strongly reduced with *Menidia* ($P = 0.0003$, Fig. 2E). Abundance of suspended filaments ($P = 0.016$, Fig. 2F) and of the diatom *Synedra* ($P = 0.034$, Fig. 2G) was elevated in *Menidia* tanks. Benthos exhibited little response to the presence of *Menidia*. Dytiscid adults ($P = 0.064$, Fig. 1D) and larvae ($P = 0.002$, Fig. 1E) and benthic *Simocephalus* ($P = 0.084$, Fig. 1F) were depressed and *Pantala* larvae ($P = 0.004$, Fig. 1C) were more abundant in *Menidia* tanks.

The presence of tadpoles had minor impacts on tank communities. In the plankton, *Diaptomus* ($P = 0.092$, Fig. 2B) densities were reduced and seston particles in the 16–32 μm range ($P = 0.090$, Fig. 3A) and dissolved oxygen ($P = 0.061$, Fig. 3B) were elevated in tadpole tanks. In the benthos, *Caenis* ($P = 0.092$, Fig. 1H) and dytiscid adults ($P = 0.014$, Fig. 1D) and larvae ($P = 0.018$, Fig. 1E) were less abundant and densities of *Pantala* ($P = 0.066$, Fig. 1C) were elevated. (For a summary of all main effects, see Table 2).

Micropterus × *Menidia* Interaction Effects

Interaction effects are responses to combinations of treatments which are not of the magnitude expected by summing the responses of the single treatments. Possible causes of interaction effects in food web manipulations include changed behaviors of one predator in the presence of another (e.g. increased vigilance) and shifts in prey selection resulting from competitive interactions. The most striking interaction effects

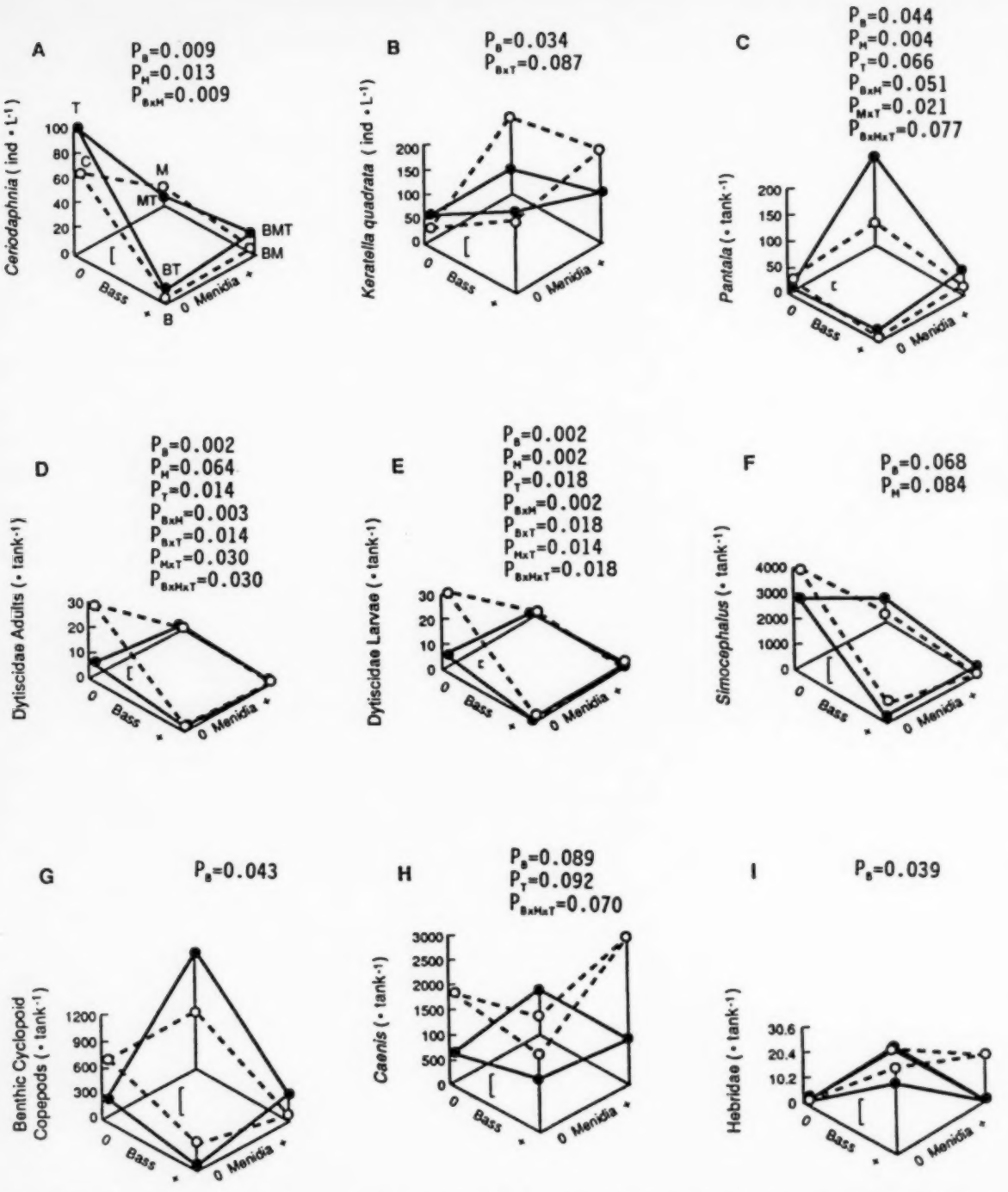

FIG. 1. Food web components responding most strongly to manipulation of *Micropterus*. Plotted points are least-square means of densities (units indicated on plot) through time with 1 SE indicated by vertical bars. (A) *Ceriodaphnia*; (B) *K. quadrata*. (C) *Pantala* naiads: (D) Dytiscidae adults; (E) Dytiscidae larvae; (F) *Simocephalus*; (G) benthic cyclopoid copepods; (H) *Caenis* nymphs; (I) Herbridae adults and nymphs. ○, treatments without tadpoles; ●, with tadpoles. Treatment combinations: B = *Micropterus*, M = *Menida*, T = tadpoles, BM = *Micropterus* with *Menidia*, BT = *Micropterus* with tadpoles, MT = *Menidia* with tadpoles, BMT = *Micropterus* with *Menidia* and tadpoles. $N = 2$ replicate tanks through time (six dates). Probability statements are results of three-factor ANOVA.

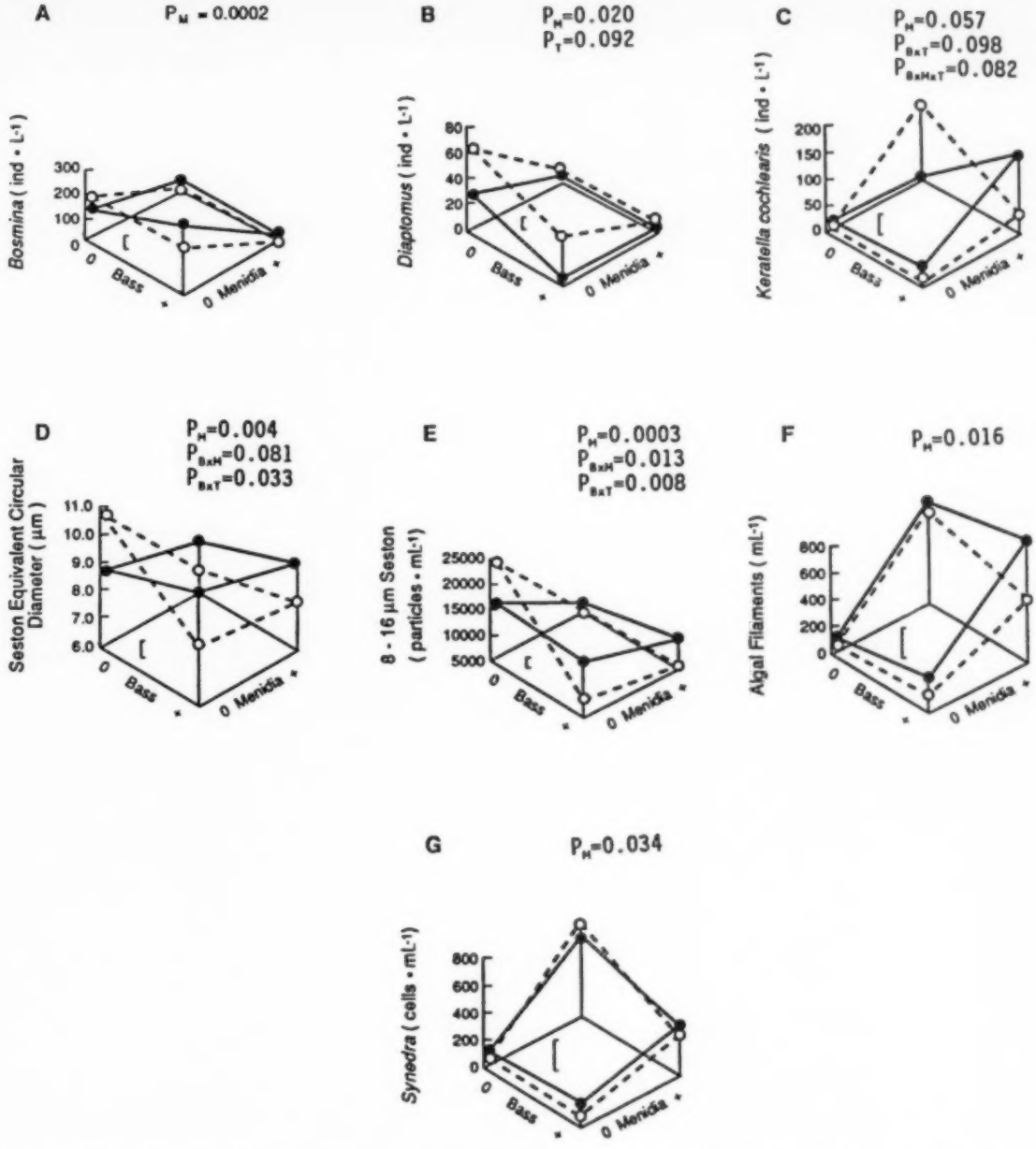

FIG. 2. Food web components responding most strongly to manipulation of *Menidia* (units indicated on plot). (A) *Bosmina*; (B) *Diaptomus*; (C) *K. cochlearis*; (D) seston particle size (equivalent circular diameter); (E) density of 8–16 µm diameter seston; (F) sestonic algal filaments; (G) *Synedra*. Graph descriptions as in Fig. 1.

in our experiment were those in which the presence of both fish together caused responses greater than the sum of the responses in the presence of either fish alone with respect to controls. This pattern was seen with *Eurytemora* ($P_{B\times M}$ = 0.057, Fig. 4A), copepod nauplii ($P_{B\times M}$ = 0.026, Fig. 4B), odonate emergence ($P_{B\times M}$ = 0.048, Fig. 4C), and diptera pupae ($P_{B\times M}$ = 0.062, Fig. 4E).

A second pattern is seen where the response to the presence of both fish was less than additive but still positive relative to controls. This was seen most commonly in measures of seston size and abundance: particle size (equivalent circular diameter, $P_{B\times M}$ = 0.081, Fig. 2D) and density of particles in the range of 8–16 µm ($P_{B\times M}$ = 0.013, Fig. 2E).

A third pattern emerges where the response variable was reduced to a level less than that predicted from the effect of either fish alone (negative relative to controls). *Ceriodaphnia* densities exhibited this pattern ($P_{B\times M}$ = 0.009, Fig. 1A). Here, it appears that the loss of *Ceriodaphnia* from the *Microp-*

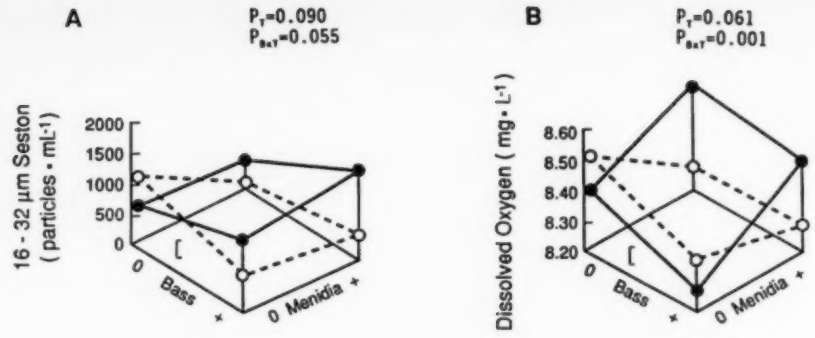

FIG. 3. Food web components responding most strongly to manipulation of *Menidia* (units indicated on plot). (A) Density of 16–32 µm seston; (B) dissolved oxygen. Graph descriptions as in Fig. 1.

TABLE 2. Summary of main effects of a *Micropterus*, *Menidia*, tadpole manipulation. + = elevated relative to controls; − = reduced relative to controls.

Variable	*Micropterus*	*Menidia*	Tadpoles
Algal filaments		+	
Synedra sp.		+	
Seston particles			
8–16 µm[a]		−	
16–32 µm[a]			+
Cladocera			
Bosmina		−	
Ceriodaphnia	−	−	
Copepoda			
Diaptomus		−	
Rotifera			
Keratella cochlearis		+	
Keratella quadrata	+		
Ephemeroptera			
Caenis	+		−
Hemiptera			
Corixidae	−	−	
Notonectidae	−	−	
Hebridae	+		
Coleoptera			
Dytiscidae (adults)	−	−	−
Dytiscidae (larvae)	−	−	−
Odonata			
Pantala	−	+	+
Benthic cyclopoid copepods	−		
Simocephalus	−	−	

[a]Longest dimension.

terus tanks was accelerated in the presence of *Menidia* and vice versa. The occurrence of aborted odonate emergence attempts (death of individuals while in emergence stage 2: Corbet 1963) ($P_{B\times M}$ = 0.047, Fig. 4D) and the numbers of *Pantala* (odonate) larvae ($P_{B\times M}$ = 0.051, Fig. 5C) also showed this pattern.

Micropterus × Larval *Hyla* Interactions

The first pattern of interactions (the combination of both animals together had a greater than additive effect on the response variable) was the most commonly observed pattern of interaction effects between *Micropterus* and *Hyla* larvae. Seston particles of 8–16 µm ($P_{B\times T}$ = 0.008, Fig. 2E) and 16–32 µm ($P_{B\times T}$ = 0.055, Fig. 3A) size ranges, particle size (equivalent circular diameter, $P_{B\times T}$ = 0.033, Fig. 2D), and algal fluorescence ($P_{B\times T}$ = 0.058, Fig. 5A) exhibited this pattern. Of the zooplankton, only *K. cochlearis* responded to the combination of *Micropterus* and *Hyla* ($P_{B\times T}$ = 0.098, Fig. 2C). Here, *K. cochlearis* was in greatest abundance in the presence of both consumers. *Berosa* adults (Hydrophilidae: Coleoptera) also responded as in this manner ($P_{B\times T}$ = 0.030, Fig. 5B).

Periphyton ash-free dry weight ($P_{B\times T}$ = 0.094, Fig. 5C) exhibited a response where tanks with both *Micropterus* and *Hyla* developed densities intermediate to those with either treatment alone.

Only the ratio of algal fluorescence/DCMU-enhanced fluorescence exhibited an interaction where the ratio in the combination tanks was less than in either single-species tanks

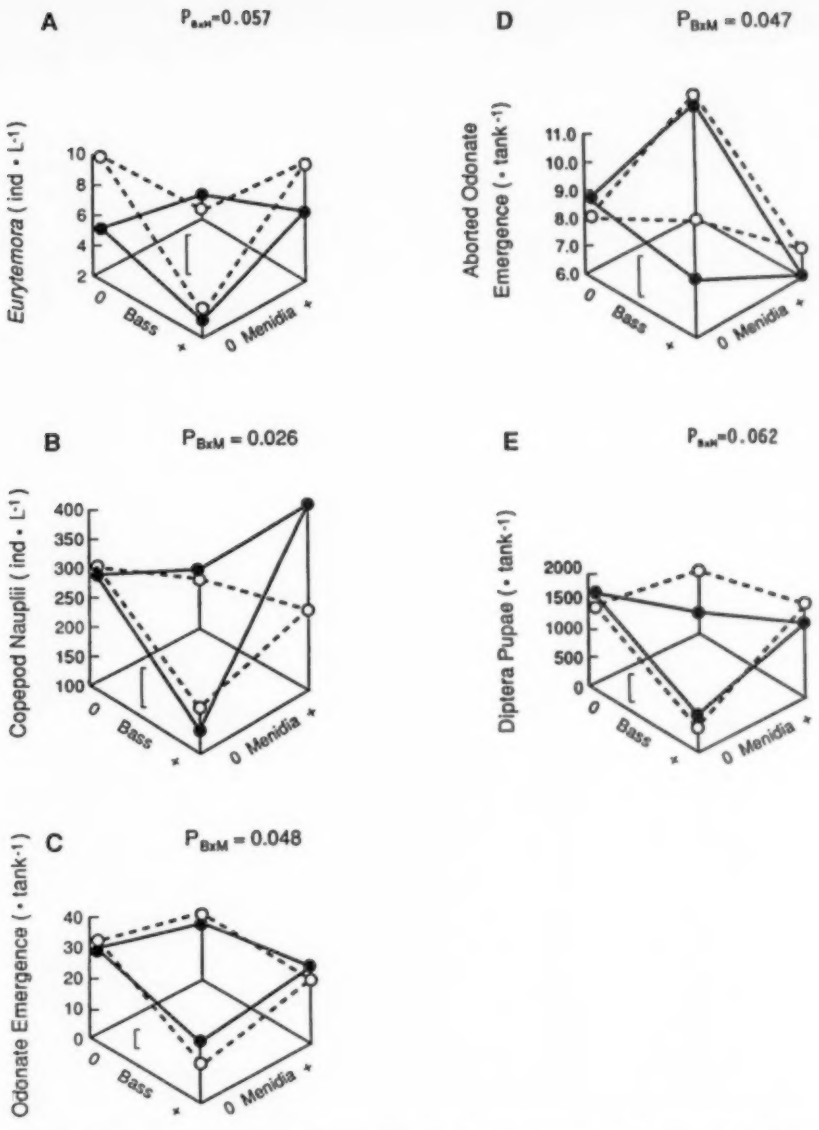

FIG. 4. Food web components responding most strongly to the presence of both *Micropterus* and *Menidia* (units indicated on plot). (A) *Eurytemora*; (B) copepod nauplii; (C) Odonate emergence; (D) aborted odonate emergence; (E) Diptera pupae. Graph descriptions as in Fig. 1.

($P_{B\times T}$ = 0.091). This pattern was the inverse of that for algal fluorescence (Fig. 5A) and indicates that the *Micropterus* + tadpole treatments resulted in "healthier" algal cells than either treatment alone (Samuelsson et al. 1978).

Menidia × Larval *Hyla* Interactions

There were few interactions associated with this treatment combination. Dissolved oxygen ($P_{M\times T}$ = 0.001, Fig. 3B), periphyton dry mass ($P_{M\times T}$ = 0.002, Fig. 5D), and *Pantala* larvae density ($P_{M\times T}$ = 0.021, Fig. 1C) exhibited the pattern where tanks containing both treatment organisms showed a greater response than either treatment alone. Densities of the rotifer *K. quadrata* ($P_{M\times T}$ = 0.087, Fig. 1B) were reduced in the presence of both *Menidia* and *Hyla* and elevated with *Menidia* alone. Dytiscid adults ($P_{M\times T}$ = 0.030, Fig. 1D) and larvae ($P_{M\times T}$ = 0.014, Fig. 1E) were at near zero densities in the presence of *Menidia* alone or *Menidia* with *Hyla*. (For a summary of all interaction effects, see Table 3.)

Unaffected Variables

Notonectids and corixids were rare in tanks with fish, relative to controls, yet due to high variance in control tanks, this effect was not statistically significant. Very common, but cryptic taxa were unaffected by fish or tadpoles (e.g. *Berosus* larvae:

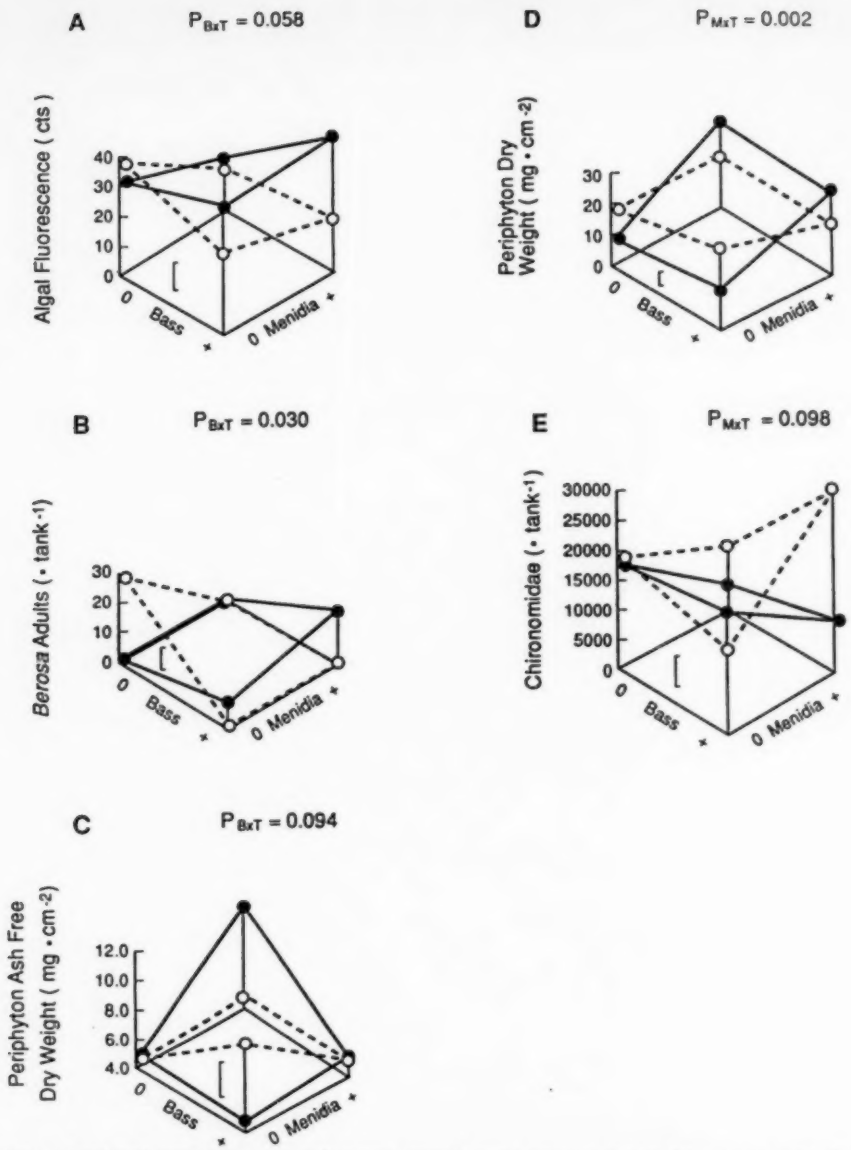

FIG. 5. Food web components responding most strongly to the presence of both *Micropterus* and tadpoles and *Menidia* and tadpoles (units indicated on plot). (A) Algal fluorescence; (B) *Berosa* adults; (C) periphyton ash-free dry weight; (D) periphyton dry weight; (E) Chironomidae. Graph descriptions as in Fig. 1.

Coleoptera: Hydrophilidae; *Ochrotrichia*: Trichoptera: Hydroptilidae; *Cyrnellus*: Polycentropidae; *Chaoborus*: Diptera: Chaoboridae; Ceratopogonidae and Oligochaeta) (Table 4). No measures of benthic biomass were affected by experimental treatments.

Density Dependence of Responses to Fish Treatments

Two relatively uncommon rotifers, *Polyarthra* and *Notholca*, showed consistently significant positive responses to elevated fish density (covariate = total fish density: $P = 0.0004$ and 0.0001, respectively) and fish mass (covariate = total fish biomass: $P = 0.018$ and 0.046, respectively). Otherwise, neither of these rotifers exhibited consistent responses to the treatments. *Diaptomus* showed negative response to increased fish mass ($P = 0.047$), but not density, in the combined fish treatment tanks. Sestonic algal filaments showed a positive response to combined fish density ($P = 0.060$) but not to fish biomass. No other variables showed significant responses to fish mass or density.

Condition and Mortality of Fish and *Hyla* Larvae

Fish mortality during the experiment was significantly affected by the presence of the other fish species. The numbers

TABLE 3. Two- and three-way interactions of a *Micropterus*, *Menidia*, and *Hyla* tadpole manipulation. B = *Micropterus*, M = *Menidia*, T = tadpoles, C = controls, BM = *Micropterus* + *Menidia*, BT = *Micropterus* + tadpoles, MT = *Menidia* + tadpoles, BMT = *Micropterus* + *Menidia* + tadpoles. Inequalities refer to variables with respect to the treatment combinations involved with the interaction.

Variable	B × M	B × T	M × T	B × M × T
Algal fluorescence		BT > C > T > B		
Seston				
8–16 μm[a]	C > B > BM > M	C > BT > T > B		
16–32 μm[a]	C > B > BM > M	C > BT > T > B		
Cladocera				
Ceriodaphnia	C > M > B > BM			
Copepoda				
Eurytemora	C > B > BM > M			
Nauplii	C > BM > M > B			
Rotifera				
Keratella cochlearis		BT > T > B > C		
Keratella quadratus			M > T = MT > C	
Diptera				
Chironomidae			M > T > C > MT	
Diptera pupae	C > BM > M > B			
Coleoptera				
Berosa adults		C > BT > B = T		
Dytiscidae adults	C > M > B = BM	C > T > B = BT	C > T > M = MT	C > T > M > B = BT = BM = MT = BMT
Dytiscidae larvae	C > M > B = BM	C > T > B = BT	C > T > M = MT	C > T > M = B = BT = BM = MT = BMT
Odonata				
Pantala	M > C > BM > B		MT > M > T > C	
Odonate emergence	C > BM > M > B			
Aborted emergence	M > B > C > BM			
Periphyton biomass (g dry wt)			MT > C > M > T	

[a]Longest dimension.

of *Micropterus* and *Menidia* recovered from tanks with both fish (Table 5) were significantly reduced compared with tanks with a single fish species (Table 6). The presence of tadpoles had no significant effect on the number of fish recovered.

Menidia recovered from *Micropterus* tanks were larger and in better condition (k) than those recovered from other tanks (Tables 5 and 6). Total biomass (grams per square metre) of *Micropterus* recovered from *Menidia* tanks was less than that of other tanks (Table 5).

The presence of tadpoles most affected *Micropterus*. *Micropterus* recovered from tadpole tanks were significantly larger than those from other tanks (Tables 5 and 6). A significant *Menidia* × tadpole interaction indicated that total length, dry weight, and condition of *Micropterus* were greater in the presence of both *Menidia* and tadpoles than in that of either alone. *Menidia* total dry weight (grams per square metre) was enhanced in tanks with tadpoles and the combination of *Micropterus* and tadpoles.

All tadpoles metamorphosed or were lost from the tanks before the end of the experiment and no data on relative mass change are available.

Discussion

Fish Effects

Menidia affected food web structure more strongly than did *Micropterus* in our experiment. In the presence of *Menidia*, measures of autotrophic standing stock (algal fluorescence and sestonic filament mass) were elevated above controls. Near extinction of cladoceran algal grazers in *Menidia* tanks suggests that intense planktivory may have been responsible for the increase in autotrophs in the *Menidia* tank communities. This was not so for *Micropterus*. The presence of *Micropterus* resulted in the reduction in abundance of *Ceriodaphnia*, a common algal grazer, but the additional reduction of *Bosmina* by *Menidia* apparently was necessary to release phytoplankton from grazer control to levels detectable in this experiment. Lack of response of *Bosmina* densities to the presence of *Micropterus* may have been due simply to *Bosmina* being too small or cryptic to be susceptible to capture by *Micropterus* (Zaret and Kerfoot 1975; Zaret 1980).

These data support the findings of food web studies (Menge and Sutherland 1976; Pimm and Rice 1987; Vadas 1990) that suggested that the impacts of omnivory (e.g. *Micropterus*) are less disruptive and destabilizing to the surrounding food web than the impacts of specialized feeding. Although results of early food web models (e.g. Pimm 1982) suggested that omnivory is rare and destabilizing, recent analysis of more complete food webs in a wide range of habitats points to the opposite conclusions (e.g. Spataru and Gophen 1985; Donaghay 1988; Sprules and Bowerman 1988; Warren 1989; Vadas 1990; Hall and Raffaelli 1991; Polis 1991). In our experiment, there was little evidence of depletion or even significant reduction of potential prey in *Micropterus* (omnivore) tanks. Nearly complete elimination of algal-grazing zooplankton in *Menidia* tanks illustrated the potential for a trophic specialist to alter a food web under these experimental conditions. The relatively short duration of our experiment probably precluded the development of extreme resource fluctuations, characteristic of destabilized food webs (Pimm 1982). Because we had no gut data, we have relied on numerous

TABLE 4. Treatment least-square means of variables not showing significant response to the treatments. $MSE^{0.5}$ = root mean square error, Cont. = control, B = *Micropterus*, M = *Menidia*, T = tadpoles, B × M = *Micropterus* + *Menidia*, B × T = *Micropterus* + tadpoles, M × T = *Menidia* + tadpoles, B × M × T = *Micropterus* + *Menidia* + tadpoles. n = 2 replicate tanks through time (six dates).

		ANOVA least-square means							
Variable	$MSE^{0.5}$	Cont.	B	M	T	B × M	B × T	M × T	B × M × T
Seston (μm)									
2–4	1763.7	7634	7086	8441	8813	8124	8974	7114	8951
4–8	2680.1	12976	10494	10496	14146	9760	13698	94611	4517
32–64	39.5	122	151	95	108	152	131	128	104
64–12	9.4	0	20	14	7	7	17	10	10
Circular diameter	0.3	2	2	2	2	2	2	2	2
Blue-green filaments (no.·mL^{-1})	270.6	20	45	78	27	158	74	135	581
Zooplankton (no.·L^{-1})									
Cyclops sp.	12.5	15	22	6	3	22	9	8	19
Brachionus sp.	4.9	1	1	3	0.3	8	2	3	6
Notholca sp.	19.9	0.4	40	3	5	6	3	4	5
Polyarthra sp.	8.7	1	19	2	0.1	8	3	3	6
Benthic invertebrates (no.·tank^{-1})									
Ceratopogonidae	78.7	0	154	43	37	5	0	0	55
Chaoborus	572.1	1102	0	28	0	0	257	297	19
Tanypodinae	373.2	505	944	735	836	899	415	846	638
Corixidae	20.5	65.2	0	0	0	0	0	0	0
Notonectidae	104.3	208.2	0	0	5.7	0	0	0	0
Cyrnellus	103.4	122	93	221	148	154	69	65	163
Ochrotrichia	91.1	175	10	28	31	18	9	16	0
Oligochaeta	4433.5	1308	0	626	522	134	250	1158	8785
Alona	500.3	0	0	0	0	5	0	1001	55
Pelycepoda	16.3	0	0	0	0	9	0	31	0
Gastropoda	145.1	257	0	2	46	5	126	0	0
Benthic organic matter (g·tank^{-1})									
Ash-free dry wt	185.8	623	375	719	421	611	551	558	542
Total ash	96.5	339	203	377	443	299	318	248	318
Total dry wt	214.0	962	579	1096	864	910	869	806	862
Benthic filament dry wt	16.5	0	18	27	17	14	1	19	2
Allochthonous leaf mass	3.3	7	6	7	9	5	6	9	9
Physical data									
Temperature (°C)	0.5	28	28	28	27	28	28	27	28
Turbidity (NTU)	0.3	2	2	1	2	1	2	2	2
Secchi depth (cm)	11.8	167	178	174	167	178	169	180	157

TABLE 5. Initial and recovery data on fish used in fish treatments (as means, n = 2). B = *Micropterus*, M = *Menidia*, T = *Hyla* tadpoles. See Table 4 for treatment definitions.

	Menidia				*Micropterus*			
	M	B × M	M × T	B × M × T	B	B × M	B × T	B × M × T
Initial no.	20	20	20	20	10	10	10	10
No. recovered	10.5	5.5	16	8	7.5	3.5	7	3.5
Total length	6.8	8.5	6.7	7.4	5.7	5.1	5.8	6.2
Standard length	5.5	7.5	6	6.9	4.8	4.3	4.9	5.3
Initial wt (g·m^{-2})	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Final wt (g·m^{-2})	0.9	1	1.8	1.3	0.7	0.5	0.7	0.4
Dry wt (g·fish^{-1})	0.3	0.7	0.5	0.7	0.5	0.3	0.4	0.6
Condition (k)	0.17	0.14	0.17	0.12	0.25	0.21	0.23	0.24

food habit studies to indicate that organisms in our tanks were potential prey (e.g. Saunders 1959; Keast et al. 1978; George and Hadley 1979; Hamilton and Prowles 1983).

Micropterus of the size used in this experiment (6–10 cm total length) appeared to prey upon and compete with *Menidia* (from observations of *Menidia* in *Micropterus* mouths and from data on fish mass at recovery). The effectiveness of *Micropterus* as a predator on *Menidia* was seen in the significant reduction in numbers of *Menidia* recovered at the end of the experiment. *Menidia* larger than 8.5 cm total length were relatively immune to predation. Once the susceptible *Menidia* were captured by *Micropterus*, the remaining *Menidia* were able to utilize food

TABLE 6. Probability values on *F*-tests from two-factor ANOVA for fish recovery variables. ns = $P > 0.1$, + = enhancement, − = suppression.

	Menidia in the presence of:			*Micropterus* in the presence of:		
Response	*Micropterus*	Tadpoles	*Micropterus* + Tadpoles	*Menidia*	Tadpoles	*Menidia* + Tadpoles
No. recovered	0.002 −	ns	ns	0.002 −	ns	ns
Total length	0.031 +	ns	ns	ns	0.001 +	0.005
Standard length	0.0001 +	ns	0.092	ns	0.001 +	0.025
Final dry wt ($g \cdot m^{-2}$)	0.078 +	0.003 +	0.040	0.066 −	ns	ns
Ind. dry wt ($g \cdot m^{-2}$)	0.0001 +	ns	ns	ns	0.010 +	0.0003
Condition (*k*)	0.002 +	ns	ns	ns	ns	0.010

resources more effectively than *Micropterus*, resulting in increased condition of *Menidia* and reduced weight and number of *Micropterus* recovered. Prey that could probably be utilized by both species of fish but were more susceptible to *Menidia* included *Ceriodaphnia*, *Eurytemora*, and small odonates. Intense competitive interactions of this sort have been shown to lead to reduced recruitment or shifts in timing of spawning in either species over time (Cryer et al. 1986). A model of size–time dependent interactions among young *Micropterus*, *Dorosoma petense*, and *D. cepedianum* (Adams and DeAngelis 1987) indicates that young *Micropterus* depend on the availability of vulnerable-sized prey to guarantee sufficient growth for overwinter survival. Nothing is known about how *Micropterus*, with inadequate piscine prey, switch to alternate prey and what impact such a switch would have on other prey such as benthic macroinvertebrates and large zooplankton. Nor is it clear what impact the competitive interaction between obligate invertivores (zooplanktivores and benthivores) and young piscivores eating invertebrates has on the invertivores.

While the small spatial scale and habitat homogeneity of the experimental tanks precluded predator-mediated habitat segregation and resource (prey) avoidance (sensu Power 1984; Power et al. 1985; Harvey 1991), behavior of *Menidia* was different in the presence of *Micropterus*. We often observed *Micropterus* pursuing *Menidia*. Changes in behavior by *Menidia* (e.g. increased vigilance and *Micropterus* avoidance) may account for elevated densities of potential *Menidia* prey (*Eurytemora* and *Caenis*) in tanks containing both fish. DeVries (1990) and Gotceitas and Colgan (1990) have shown, experimentally, that *Lepomis* changes its foraging behavior in the presence of *Micropterus*, resulting in reduced feeding success and elevated *Lepomis* prey abundance.

Tadpole Effects

Tadpoles in our study exhibited little ability to regulate key elements of these food webs (e.g. algal biomass and zooplankton densities). Hambright et al. (1986) also found that *Rana catesbeiana* tadpoles had no detectable effects on phytoplankton in experimental ponds with *Lepomis* and *Micropterus*. In naturally fishless ponds, amphibian larvae are considered to act as "regulatory consumers" (Seale 1980). At high densities (Seale 1980: approximately $30 \cdot m^{-2}$; Osborne and McLachlan 1985; $870–3500 \cdot m^{-2}$), amphibian larvae can regulate primary production via ingestion of particulate organic nitrogen and conversion to dissolved organic and inorganic nitrogen. In our study, we used tadpole abundances of $19 \cdot m^{-2}$, apparently too low to act in a regulatory manner.

Tadpoles, by themselves, had little effect on any of the planktonic components of the tank food webs. Odonates, which prey on tadpoles (Caldwell et al. 1980; Travis et al. 1985), were in greater abundance in the presence of tadpoles than in controls, probably as a result of greater larval survival. *Caenis* larvae were slightly suppressed by tadpoles, possibly by way of competitive interference during feeding on periphyton. This outcome was opposite to that reported by Morin et al. (1988) with *H. andersonii* and a suite of grazing insects, where metamorphs showed a reduction in mass in the presence of the grazers.

Despite the lack of strong main effects associated with the tadpole treatment, there was evidence that tadpoles played a key role in the indirect enhancement of primary, secondary, and tertiary producers. Enhancement of algal fluorescence and several seston particle size classes, increased odonate densities, and enhanced survivorship of *Micropterus* in tadpole treatments suggest that a wide range of interactions were mediated by the periphyton-grazing amphibian larvae.

Fish–Tadpole Interactions

Despite the relatively short residency of *Hyla* in the fish tanks, a large number of interactions with fish were detected. Periphyton mass was enhanced in the presence of both tadpoles and *Menidia*. Without *Menidia*, the grazing of tadpoles appeared to be strong enough to suppress periphyton mass, and in *Menidia* tanks without tadpoles, periphyton growth was not enhanced.

The presence of both tadpoles and *Menidia* also had a positive effect on the density of *Pantala* larvae. Survival of *Pantala* in these tanks was probably enhanced by increased physical structure (periphyton) which provides odonates a good habitat from which to capture prey (Johnson and Crowley 1980), as well as protection from fish predation (Crowder and Cooper 1982).

Elevated algal fluorescence, and densities of seston and *Keratella cochlearis* in the presence of *Micropterus* and tadpoles, suggests that grazing by tadpoles possibly released nutrients contained in periphyton into the water column. It is also likely that nutrients contained in tadpole biomass were released as a result of *Micropterus* predation (Hamilton and Prowles 1983; Hambright et al. 1986). Osborne and McLachlan (1985) showed that tadpoles play a major role in transforming sediment-bound nutrients into dissolved forms usable by phytoplankton. Tadpole-mediated enhancement of algal production was not apparent in the tadpole-only treatments of our experiment, possibly because nutrients were exported from the tanks via metamorphosis and emergence of tadpoles as frogs (Seale 1980). The presence of fish was necessary for tadpole effects to be seen.

Tadpoles may have acted to offset the competitive effects of the fish on each other by acting as an alternative food source (in *Micropterus* tanks) or by indirectly enhancing resource levels (in *Menidia* tanks). *Micropterus* grew better in the tanks with tadpoles than in the other tanks. The cause of elevated weight and numbers of individuals of *Menidia* recovered from tadpole tanks is not clear.

Fish–Benthos Effects

Our data show strong evidence that young *Micropterus* and adult *Menidia* suppressed abundance of large-bodied, active invertebrates. Small-bodied taxa were relatively unaffected by the presence of either fish. We can only assume that the depression of invertebrate density was the result of fish predation because the guts of fish harvested at the end of the experiment were nearly all empty or contained only detritus. However, reduction of particular invertebrate groups in fish tanks follows patterns seen in studies where fish guts were examined (Saunders 1959; Hall et al. 1970; George and Hadley 1979; Hamilton and Prowles 1983). Shifts to smaller taxa, and smaller individuals within a taxon, have been observed in the presence of various predatory fish studies: bluegill (*Lepomis macrochirus*) (Hall et al. 1970; Werner and Hall 1974), yellow perch (*Perca flavescens*) (Post and Cucin 1984), *Gambusia* (Hurlbert et al. 1972), and *Cyprinus* and *Menidia* (Richardson et al. 1990). The exceptions to this pattern in our experiment were the elevated densities of *Caenis* with *Micropterus* and *Pantala* with *Menidia*. Hall et al. (1970) noted that *Caenis* densities were elevated in ponds with bluegill and suggested that a selective removal of invertebrate predators by fish caused the *Caenis* response. This may also be the case in our study.

Increased densities of *Pantala* in *Menidia* tanks may have been the result of gape-limited feeding by *Menidia*. *Menidia* diets in Lake Texoma consist primarily of large zooplankton and insects smaller than most odonates (Saunders 1959). Odonate larvae may have avoided *Menidia* predation by rapidly outgrowing vulnerability. Larvae in the tanks at the start of the experiment may have already been too large for *Menidia* to consume. It is also possible that the duration of the experiment was too short to allow oviposition and hatching of younger, smaller odonate larvae. Alternatively, increased periphyton accumulation in *Menidia* tanks may have provided refuge for odonates from fish predation. In *Micropterus* tanks, no enhancement of periphyton occurred, leaving odonates vulnerable to *Micropterus* predation (Hamilton and Prowles 1983), possibly resulting in reduced densities and emergence rates (and elevated numbers of *Caenis* mayflies).

Invertebrates may be particularly vulnerable to fish predation only during specific life stages. In this experiment, densities of Diptera pupae were reduced by *Micropterus*, while larvae were relatively unaffected. Butler (Department of Zoology, North Dakota State University, pers. comm.) found that 24–31% of the chironomids (*Chironomus cucini*) leaving the sediments of several northern lakes as pupae failed to reach the lake surface. Gut analysis of fish in these lakes indicated that much of the profundal *Chironomus* production was consumed by fish preying on pupae, not on larvae. Hall et al. (1970) found reduced chironomid emergence rates as a result of fish predation on fourth-instar larvae and pupae. Such results suggest that effects of fish predation on insects may best be considered in terms of some integrative measure such as secondary production rather than larval densities.

The conditions of relative substrate homogeneity in the tanks and spatially constrained predators should have provided an ideal situation for a clear predator effect (Crowder and Cooper 1982). Yet both species of fish had relatively minor impacts on macroinvertebrates. Stronger fish effects (e.g. reduced benthic biomass and individual size) have been shown in some manipulative studies, but only with higher fish densities and longer exposure times (Hayne and Ball 1956; Andersson et al. 1978; Crowder and Cooper 1982).

The nonadditive, multispecies effects seen in this experiment were not simply the result of increased fish biomass in the combination treatments. ANCOVA with fish mass and density detected few effects of elevated fish biomass. As suggested by Fauth (1990), nonadditivity is often the result of interrelationships (predatory and competitive) of these fish with each other and with the tank communities.

Summary and Conclusions

Experimental manipulation of three species of aquatic vertebrates, a periphyton grazer, an omnivore, and a zooplanktivore, suppressed large, active invertebrates in all treatments. Direct feeding effects in the water column were only seen in *Menidia* tanks where nearly all zooplankton were eliminated. Density of the dominant benthic macroinvertebrate group, Chironomidae, was relatively unaffected by fish or tadpoles. Under these experimental conditions, young-of-the-year *M. salmoides*, *M. beryllina*, and *H. chrysocelis* tadpoles had relatively minor effects on benthic organisms and stronger effects on zooplankton.

Many changes in the food webs occurred in the presence of two or three consumers simultaneously. These interaction effects indicate a lack of independence in response to the simultaneous presence of the two fish species and larval *Hyla*. Single fish species treatments usually caused a trajectory away from controls in one direction, while the treatments with both fish species deviated in the other direction or exaggerated the single fish species treatment effect. Densities of *Eurytemora*, *Ceriodaphnia*, copepod nauplii, seston, Diptera pupae, *Pantala* larvae, odonate emergence, and aborted emergence responded in this nonadditive pattern.

Our experiment implies that these particular food webs were not pieced together in a building-block fashion because the impact of a given consumer depended on the presence of other consumers. The predominance of interaction effects suggests that food web experiments using a single fish species may lead to incorrect inferences regarding responses of multiconsumer systems (Fauth 1990; Wilbur and Fauth 1990).

Acknowledgements

We gratefully acknowledge P. Cantrell (University of Oklahoma) for her technical support, A. Clements and Alexandra Webber (Savannah River Ecology Laboratory) for preparing the figures, and J. J. Hoover (U.S. Army Corps of Engineers) for identification of the chironomids. This research was supported by NSF grant BSR 880 6581. Reviews by two anonymous reviewers greatly improved this paper. P. Dixon (Savannah River Ecology Laboratory) provided statistical council. Manuscript preparation was supported by contract DE-AC-09-76SR00819 between the U.S. Department of Energy and the University of Georgia's Savannah River Ecology Laboratory.

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Morphometrics of American Lobster (*Homarus americanus*) Larvae in Relation to Stock Determinations in the Maritimes, Canada

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Morphological characteristics of the first larval stage of the American lobster (*Homarus americanus*) enabled the separation of the Maritime population with stepwise discriminant function analysis into three groups: the southern Gulf of St. Lawrence and its outflow around Cape Breton Island, the Atlantic inshore region of Nova Scotia, and the offshore banks bordering the Gulf of Maine. Once the effect of environmental temperature on larval size was removed, the differences between Georges and Browns banks and the Atlantic inshore disappeared. The remaining differences, chiefly in the dimensions of the second and fifth abdominal segments and the rostrum, between larvae from the Cape Breton sites and elsewhere may be due to other environmental factors or partial genetic isolation. Conservation and management practices over the past century have increased gene flow between regions, and most of this appears to be from the release of Gulf of St. Lawrence lobsters in the Gulf of Maine. A reassessment of previous studies on adult morphology, benthic movements, larval dispersal, enzyme electrophoresis, and commercial landing patterns supports the separation of the Gulf of St. Lawrence stock from the rest of the Canadian Maritimes.

D'après les caractéristiques morphologiques du homard (*Homarus americanus*) à la première phase larvaire, nous avons pu diviser en trois groupes la population de homard des Maritimes au moyen de l'analyse discriminante par échelons : la population de la partie sud du golfe du Saint-Laurent et de son écoulement le long de l'île du Cap-Breton, celle des eaux côtières de la région atlantique de la Nouvelle-Écosse et celle des bancs situés au large du golfe du Maine. Une fois exclu l'effet de la température ambiante sur la taille des larves, on n'enregistre plus de différences entre les larves du banc Georges, du banc de Browns et des eaux côtières de l'Atlantique. Les différences qui restent, principalement celle de la taille des deuxième et cinquième anneaux de l'abdomen et celle du rostre, entre les larves de la région de l'île du Cap-Breton et celles des autes endroits peuvent être attribuables à d'autres facteurs environnementaux ou à un isolement génétique partiel. Les pratiques de conservation et de gestion utilisées depuis le siècle dernier ont entraîné une augmentation du flux génétique entre les régions, et ce phénomène semble résulter principalement de l'introduction des homards du golfe du Saint-Laurent dans le golfe du Maine. Une réévaluation des études antérieures sur la morphologie des adultes, les déplacements benthiques, la dispersion des larves, l'électrophorèse des enzymes et les prises commerciales étaye l'hypothèse selon laquelle les stocks du golfe du Saint-Laurent seraient distincts des autres stocks canadiens des Maritimes.

Received January 28, 1992
Accepted July 14, 1992
(JB385)

Reçu le 28 janvier 1992
Accepté le 14 juillet 1992

Fisheries managers have attempted to regulate the harvest of a species to achieve a maximum sustainable yield (Ricker 1975; Gulland 1983). The concept of a biological stock developed as a means of identifying intraspecific units or subpopulations of a species with unique morphological, physiological, or ecological characteristics, enabling better management of these subunits of the resource (see Smith et al. 1990). This separation into stocks may have either an environmental or genetic basis, but large exchanges of animals between areas would render their separate management redundant. The present need for American lobster (*Homarus americanus*) stock identity arose from concern over high exploitation rates of inshore lobsters to a smaller size, particularly in the southern Gulf of St. Lawrence, and increased exploitation of large lobsters in the deeper waters in and adjacent to the Gulf of Maine which was begun in the mid-1950's by American trawlers (Skud and Perkins 1969) and in 1971 by the Canadian offshore trap fishery (Pezzack and Duggan 1983). The heavy exploitation of lobsters greater than 63.5 mm carapace length in the southern Gulf of St. Lawrence, close to the smallest size at which females first mature (Campbell and Robinson 1983), is probably selecting for maturity at smaller sizes (see Nelson and Soule 1987). Another concern is that excessive exploitation of the lobsters offshore in the Gulf of Maine may reduce an important source of larval recruitment to the adjacent lucrative inshore fisheries (Rogers et al. 1968; Saila and Flowers 1968, 1969; Stasko 1978; Fogarty et al. 1980; Harding and Trites 1988). It is important, therefore, to assess the relationship of animals

between all these geographic areas.

Various approaches have been used to evaluate the discreteness of lobster stocks. Adult lobsters from both inshore and offshore areas have been tagged to describe their seasonal movements (Saila and Flowers 1968; Cooper and Uzmann 1971; Uzmann et al. 1977; Fogarty et al. 1980; Briggs 1985; Campbell and Stasko 1985, 1986; Campbell 1986, 1989a, 1989b; Pezzack and Duggan 1986). Several authors have considered the importance of planktonic dispersal of lobster larvae in populating adjacent areas (Rogers et al. 1968; Dadswell 1979; Stasko and Gordon 1983; Harding et al. 1983; Harding and Trites 1988, 1989). Others have researched morphological (Saila and Flowers 1969; Campbell and Mohn 1982), physiological (Smith 1977; Russell 1980; Nelson et al. 1983; Aiken and Waddy 1989), and biochemical (Barlow and Ridgway 1971; Tracey et al. 1975; Odense and Annand 1978) traits to distinguish between lobster stocks. Even the incidence and type of lobster parasite have been investigated unsuccessfully as possible indicators of stocks (Uzmann 1970; Brattey and Campbell 1985). Finally, long-term landing trends have been examined for similarity among reporting districts as evidence for stock discreteness, the rationale being that these trends would reflect the interaction of all population parameters (Campbell and Mohn 1983; Harding et al. 1983). All of these diverse approaches to stock detection have not yet resulted in a consensus (Pezzack and Pringle 1986; Pezzack 1987).

The multivariate approach has been used to compare the external morphology of adult lobster populations from American and Canadian waters (Saila and Flowers 1969; Campbell and Mohn 1982). Studies of adult lobster morphometrics are complicated by size–frequency discrepancies between areas and strong sexual dimorphism which occurs at or several moults before the onset of maturity (Herrick 1911; Templeman 1935a; Aiken and Waddy 1980). The present approach avoids this complication by studying the larval lobster. In the present study, we examine the morphological variability of lobster larvae to increase our understanding of stock distinctions in the Canadian Maritimes in both inshore and offshore areas. Stage I lobsters were studied because they were abundant in our collections and their short duration of 2–10 d in the plankton (Templeman 1936a) enables fairly accurate determination of their origins or hatching locations.

Methods

First developmental stage lobsters were obtained from seven previous collections from study sites located in the Canadian Maritimes (Fig. 1). These study sites were amalgamated into broader geographic regions as our analysis of the data progressed. A variety of collecting gear was used, including plankton nets, neuston nets (Tremblay and Sharp 1989), and Vass–Tucker trawls (Vass 1988), but this would not have affected the size of larvae collected because of their weak rheotactic response (Ennis 1986). Only specimens in good physical condition were used in our morphometric study; otherwise, specimen choice was random. Each study site was a composite of larvae from different numbers of sampling stations to achieve adequate numbers of larvae for statistical analysis (Table 1). At the St. Georges Bay site, lobster larvae were collected over three years and examined separately. Surface temperature was recorded at the time of larval collection. A mean temperature was used in the present analysis when more than one station was used as a site sample. The range of temperature experienced by the embryos, while adhering to the swimmerets of the adult female lobster, was thought to be important in determining the size of the larvae. It was assumed for degree-day calculations that eggs were carried by the female for approximately 11 mo before hatching in July (Perkins 1971, 1972; Robichaud and Campbell 1991). The inshore lobsters were assumed to be relatively sedentary (Fogarty et al. 1980; Krouse 1980; Campbell and Stasko 1986), residing at an average depth of 20 m. Lobsters releasing larvae on Georges Bank (~75 m) and Browns Bank (~100 m) were assumed to be migratory (Cooper and Uzmann 1971; Uzmann et al. 1977; Campbell and Stasko 1985; Pezzack and Duggan 1986), arriving on the banks in May and returning to slope waters (200–600 m) in December. Average monthly bottom temperatures (~70 yr) were obtained for the above sites from Drinkwater and Trites (1987) and Petrie (1990). Based on these assumptions, degree-days were calculated to average the thermal history of the lobster embryos at the different collection sites.

Larvae were preserved in a 5% buffered formalin solution at the time of collection with the exception of the Sydney Bight larvae which were preserved later in a 70% alcohol solution. It is not known whether final preservation in ethanol alters specimen shrinkage relative to those kept in formalin (Steedman 1976). Linear measurements were taken of seven morphometric variables on each of 366 larvae (Fig. 2). All measurements were performed with a Wild stereomicroscope and recorded to the nearest 0.02 mm. Rostral lengths were measured from the tip of the rostrum to the posterior margin of the eye socket (line 1, Fig. 2). Carapace length was measured conventionally from the posterior margin of the eye socket to the posterior edge of the median dorsal line of the carapace (Wilder 1953). Maximum carapace length was measured also from the same location on the eye orbit but to the most posterior lateral edge of the carapace (line 3, Fig. 2). The depth of the second abdominal segment was measured from the dorsal surface, just prior to the inflection of the protuberance which is continuous with the posterior dorsal spine, to the ventral protuberance which, after the next moult, becomes the first pair of pleopods (line 4, Fig. 2). The length of the fifth abdominal segment was measured, on a lateral line parallel to the axis of the segment, from the anterior articulation with the fourth segment to its posterior margin, at a location between the bases of the posterior dorsal and lateral spines (line 5, Fig. 2). The length of the sixth abdominal segment was measured similarly from the anterior articulation with the preceding segment to the most posterior dimension, as seen in lateral view (line 6, Fig. 2). The depth of the sixth segment was measured perpendicular to and from the lowest point on the dorsal surface, just prior to the two dorsal spines, to the ventral surface (line 7, Fig. 2).

The mean, standard deviation, standard error, and correlations were calculated for each recorded variable at each site. To test for normality, G_1 and G_2 statistics were calculated for each variable at each site (Sokal and Rohlf 1969). Homogeneity of variances was tested using the F_{max} test and Box's M test (Sokal and Rohlf 1969).

Univariate analyses of variance (ANOVA) and stepwise discriminant analyses (DFA) were performed between the seven sites and between combinations of the regional classifications on the untransformed variables. The DFA's were calculated based on a criterion of maximizing the Mahalanobis D^2 between the closest pair of sites using the DISCRIMINANT subprogram of SPSS^x (SPSS Inc. 1986). To test for differences within a site, between samples collected over different years, the St. Georges Bay samples were examined using univariate ANOVA and stepwise DFA.

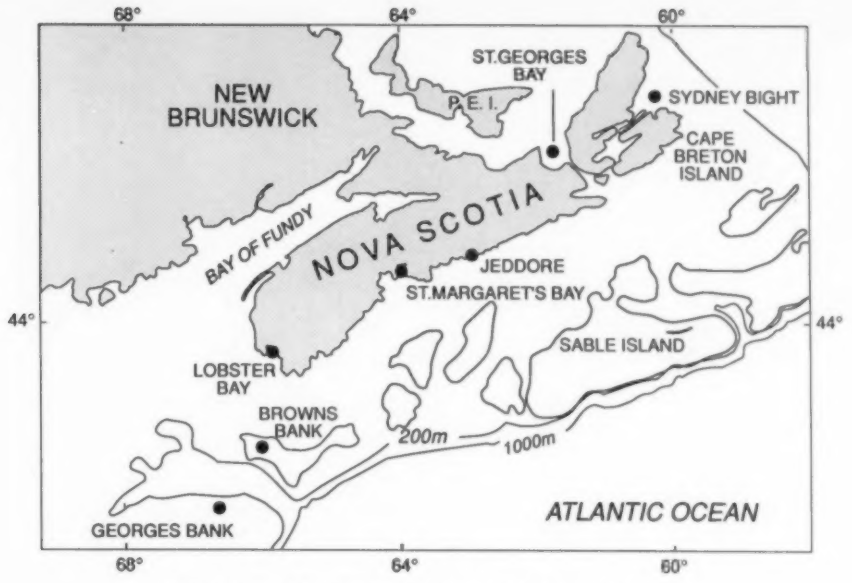

FIG. 1. Larval lobster collection sites used in the present study.

TABLE 1. Larval lobster collection data.

Site	Date	No. of stations	No. of larvae	Average temperature (°C)	Source
St. Georges Bay	July 1980	2	36		G. C. Harding, BIO[a]
	July 1985	3	17	15.1	T. L. Lambert, BIO[a]
	July 1986	2	17		T. L. Lambert, BIO
Sydney Bight	July 1978	1	43	14.5	R. J. Miller, Hfx.[b]
Jeddore Harbour	August 1988	3	23	16.4	Dibacco and Pringle 1991
St. Margaret's Bay	July 1983	23	71	16.4	J. D. Pringle, Hfx.[b]
Lobster Bay	July 1983	20	44	15.2	Tremblay and Sharp 1989
Browns Bank	July 1986	17	47	11.2	G. C. Harding, BIO
Georges Bank	July 1986	2	68	12.2	G. C. Harding, BIO

[a]Bedford Institute of Oceanography, P.O. Box 1006, Dartmouth, N.S. B2Y 4A2, Canada.
[b]Halifax Fisheries Laboratory, 1707 Lower Water Street, Halifax, N.S. B3J 2S7, Canada.

Each of the morphological variables was regressed against mean site sea temperature using the subprogram REGRESSION of SPSS[x] (SPSS Inc. 1986). Residuals from the regression line were extracted and provided a temperature-independent character suite. This data set was analyzed using ANOVA and stepwise DFA's to compare within-site and interregional differences in morphology.

Results

The morphometric characteristics measured in Stage I lobster larvae were normally distributed with the exception of the depth of the second abdominal segment in the St. Margaret's Bay sample, the length of the fifth abdominal in the Browns Bank sample, and the depth of the sixth abdominal segment in the Georges Bank sample (Table 2). Only the variance of the rostrum length character was heterogeneous between sites. The Box M test was not significant at the $P = 0.01$ level in all of the tests.

Correlation matrices of the morphometric characters from the seven geographic sites indicated the presence of significant correlations between all variable pairs, but r^2 values were low and showed no strong trends. Most correlations were less than 0.50 with the exception of carapace length versus maximum carapace length which had an r^2 of 0.79.

Differences in morphometric variables found between the three years of sampling in St. Georges Bay were not significant, using univariate ANOVA, with the exception of the depth of the second abdominal segment and the length of the sixth abdominal segment characters ($P = 0.03$). After DFA the a priori classification based on sampling year was poor (Table 3). Only the 1985 year-class was correctly classified in a large proportion (71%) of the larvae. In subsequent analyses, the three years' data from St. Georges Bay were grouped together.

A univariate ANOVA of the untransformed variables resulted in significant differences between the seven sites in all characters. A stepwise DFA showed that five characters were required to distinguish between sites (Table 4). The length of the fifth abdominal segment and the depth of the second abdominal segment were most important in distinguishing between geographic sites. The first axis accounted for 65% of the variation in the data set and effectively separated the Lobster Bay, Jeddore Harbour, and St. Margaret's Bay sites from the off-

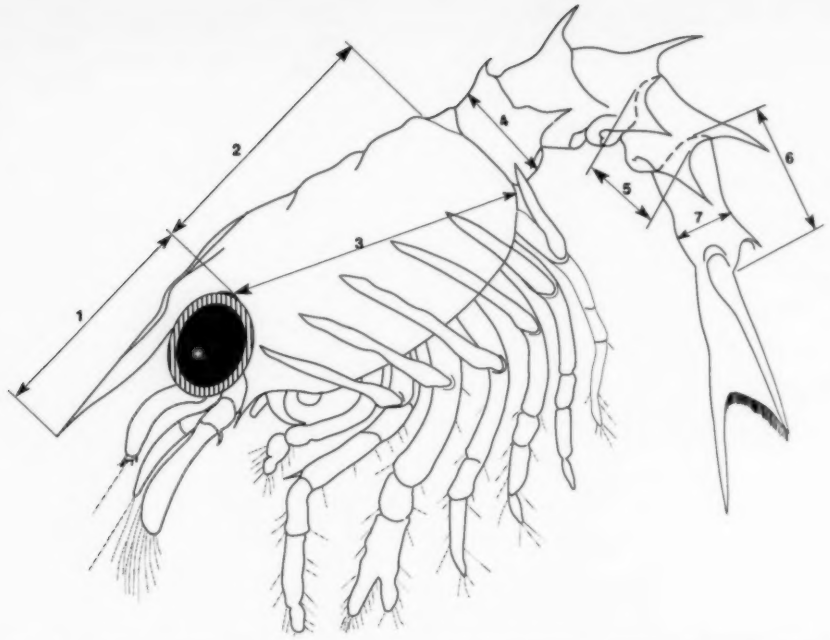

FIG. 2. First-stage lobster larvae showing the seven morphological variables measured: (1) rostral length, (2) standard carapace length, (3) maximum carapace length, (4) depth of second abdominal segment, (5) length of fifth abdominal segment, (6) length of sixth abdominal segment, and (7) depth of sixth abdominal segment.

TABLE 2. Means and standard deviations of linear morphological variables.

		Carapace			Abdominal segments			
Site	Year	Standard	Maximum	Rostrum	Depth of second	Length of fifth	Length of sixth	Depth of sixth
St. Georges Bay	1980	1.97±0.13	2.34±0.13	1.89±0.11	0.74±0.07	0.66±0.06	0.79±0.05	0.44±0.04
	1985	2.00±0.11	2.31±0.13	1.87±0.15	0.70±0.05	0.65±0.06	0.78±0.04	0.42±0.05
	1986	2.01±0.10	2.33±0.10	1.83±0.09	0.74±0.05	0.68±0.05	0.82±0.05	0.43±0.05
Sydney Bight	1978	1.99±0.16	2.33±0.14	1.88±0.13	0.75±0.05	0.66±0.06	0.80±0.04	0.43±0.03
Jeddore Harbour	1988	1.95±0.12	2.31±0.15	1.92±0.12	0.77±0.06	0.60±0.05	0.76±0.04	0.41±0.05
St. Margaret's Bay	1983	1.97±0.13	2.31±0.13	1.91±0.11	0.77±0.08	0.62±0.05	0.78±0.05	0.43±0.04
Lobster Bay	1983	1.95±0.12	2.30±0.13	1.91±0.11	0.78±0.06	0.59±0.06	0.78±0.06	0.42±0.05
Browns Bank	1986	2.03±0.12	2.39±0.11	1.95±0.09	0.81±0.05	0.69±0.05	0.81±0.04	0.46±0.04
Georges Bank	1986	2.04±0.11	2.40±0.08	1.98±0.10	0.82±0.05	0.69±0.05	0.81±0.05	0.47±0.04

TABLE 3. Separation of the three year's sampling in St. Georges Bay (percent correct classification) from DFA of the larval morphometric characters.

		Predicted group membership (%)		
Year	No. of larvae	1980	1985	1986
1980	36	47.2	30.6	22.2
1985	17	11.8	70.6	17.6
1986	17	29.4	17.6	52.9

shore bank sites (Fig. 3). The greater dimensions of the fifth and sixth abdominal segments distinguished the Georges and Browns Bank larvae from the rest. The second axis accounted for 32% of the variation and further distinguished the two Cape Breton sites of Sydney Bight and St. Georges Bay from the rest. Larvae from these sites had smaller second abdominal segment depth and rostrum length than did larvae from the Atlantic coastal waters and offshore bank sites. Larvae were assigned to one or other of these three regions based on the above analysis. In a stepwise DFA of regional groups the larvae from the offshore banks were classified correctly 80% of the time (Table 5). Correct classification of the Atlantic and Cape Breton inshore regions was also very high, although there was slightly more mixing of individuals between the groups (Table 5). The grouping of 12% of the larvae from offshore banks in the Cape Breton inshore region suggests that this level of misclassification can be considered the background variability.

The largest larval lobsters occurred in offshore waters over Georges and Browns banks (Table 2). The carapace sizes of larvae from offshore banks were significantly larger than those measured for inshore sites (t-test, $P < 0.05$). In this study the

TABLE 4. DFA of larval lobster morphometric characters from seven sites around the Maritimes. Functions shown are significant ($P < 0.05$).

Variable	Function 1	Function 2
Length of fifth abdominal segment	0.917	−0.423
Depth of sixth abdominal segment	0.468	0.035
Depth of second abdominal segment	−0.051	0.883
Length of sixth abdominal segment	−0.219	−0.126
Rostrum length	0.125	0.359
Percent of variance explained by function	65.4	32.2
Significance of function	<0.00	<0.00
Canonical correlation	0.62	0.48

morphometric variables were inversely related to the average site sea surface temperature recorded at the time of larval collection (Table 6) and positively related to average or cumulative bottom temperatures experienced during embryonic development, the latter expressed as degree-days above 3.5°C (Table 7).

To remove the effect of local temperature differences on larvae size, the residuals from bottom degree-day temperature regressions were analyzed by DFA on all seven sites, with accompanying ANOVA's. The latter demonstrated that there was a significant difference between sites in the residuals of four morphometric characters. These characters were rostrum length, the depth of the second and sixth abdominal segments, and length of the fifth abdominal segment. Discriminant function analysis of residual data after the temperature regression correction indicated a significant difference between site centroids; most of this intersite separation was achieved on the first axis (Table 8; Fig. 4). The first function was most influenced by the depth of the second and the length of the fifth abdominal segments; however, four characters were required for this discrimination. The first function served to separate the two Cape Breton sites from the others but particularly Jeddore Harbour and Lobster Bay. Browns Bank, Georges Bank, and St. Margaret's Bay formed a loose aggregation. The strong geographical separation achieved with the preceding analysis was no longer evident, apart from the continued separation of the Cape Breton sites from the others (Fig. 4). Among the Atlantic water sites the greatest variation was between Browns Bank and Lobster Bay. This separation was based primarily on the length of the fifth abdominal segment on the second axis. A separate DFA between the offshore bank region and the Atlantic inshore region showed no significant differences between these two regions. Only the Cape Breton inshore region could be seen as distinctive based on the temperature-independent data. In the final analysis, this region was compared with the remainder of the data set, referred to as Atlantic waters. The same four characters, depth of the second and sixth and length of the fifth abdominal segments and rostrum length, were significant in separating the above two regions. Discriminant function analysis indicated a significant difference between Cape Breton inshore and Atlantic waters centroids based on three characters. The first function explained all the variability and was most influenced by the depth of the second and length of the fifth abdominal segment. A correct separation was achieved in 75% of the cases between the two areas (Table 9).

Discussion

Multivariate approaches have been used previously to compare adult morphology of lobster populations in both American and Canadian waters (Saila and Flowers 1969; Campbell and Mohn 1982). In these studies the size range of lobsters examined was restricted and the sexes were treated separately in an attempt to reduce discrepancies in size and sexual dimorphism between areas. Saila and Flowers (1969) were able to distinguish lobster populations in Narragansett Bay, R.I. from those offshore in Veatch Canyon with stepwise DFA on logarithmic transformations of 16 morphological characters. Campbell and Mohn (1982) used 15 morphological variables, expressed as ratios, to differentiate Canadian lobster populations in Northumberland Strait and Cape Breton Island from those further

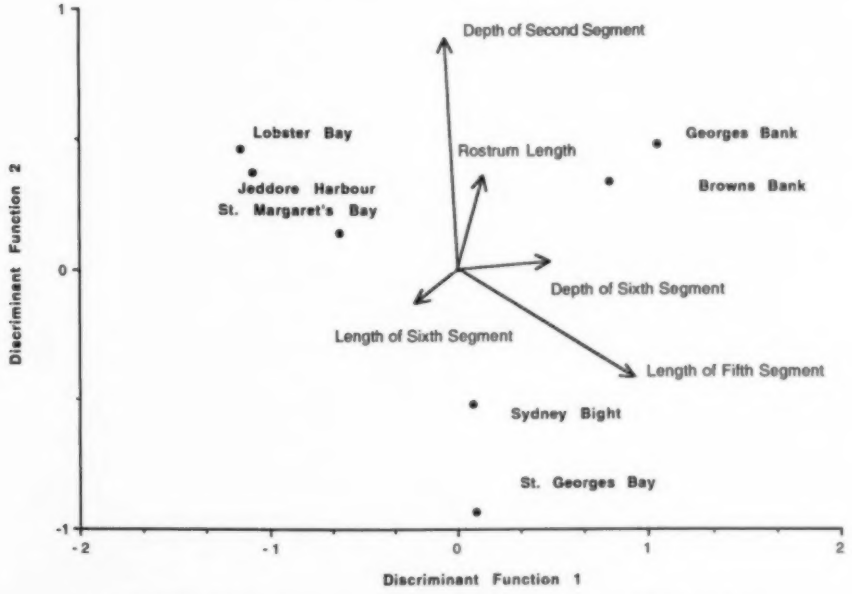

FIG. 3. DFA of lobster larvae morphometric variables from seven Maritime sites.

TABLE 5. Separation of three regions in the Maritimes (percent correct classification) by DFA of the larval morphometric characters.

Region	No. of larvae	Predicted group membership (%) Offshore banks	Atlantic inshore	Cape Breton inshore
Offshore banks	115	80.0	7.8	12.2
Atlantic inshore	139	12.3	73.2	14.5
Cape Breton inshore	113	15.0	21.2	63.7

south, including offshore regions bordering on and in the Gulf of Maine. Nevertheless, it is thought that the results of both studies were strongly influenced by secondary sexual characteristics because the size range chosen corresponds to the maximum change in the proportions of these characters, several of which were important in the conclusions reached. Furthermore, it has long been known that the onset of sexual dimorphism in lobsters occurs at different body sizes depending on the geographic locality (Templeman 1936b, 1944a). The part played by heredity in this geographic variation is unknown. The environment, in the form of summer temperatures, has an important effect on the size at which maturity occurs (Templeman 1936b) and this, in turn, determines the degree of sexual dimorphism at size. Precocious lobsters in the warmer waters of both Narrangansett Bay and the southern Gulf of St. Lawrence, together with the preponderance of larger lobsters in offshore waters, could account for a large amount of the differences observed in the above two studies.

The present approach avoids this interactive complication of size and sexual dimorphism by studying the larval lobster. First-stage larvae collected from both Georges and Browns banks were significantly larger than those from inshore sites (Table 2). This supports the earlier findings of Rogers et al. (1968) that first moult stage from Block Island and Rhode Island Sound were statistically smaller than those collected offshore over the continental shelf. MacKenzie (1985, 1988) found that there was no difference in size between stage I lobsters cultivated from captive females taken from Northumberland Strait and off southwestern Nova Scotia. Our field results indicated that stage I lobsters from St.Georges Bay, eastern Northumberland Strait, were not significantly different in size from larvae collected in Lobster Bay, off southwest Nova Scotia, or at various sites along the Atlantic coast of Nova Scotia (Table 2).

Larval lobsters were separated on morphometric grounds into three geographic regions: the southern Gulf of St. Lawrence and its outflow around Cape Breton, the Atlantic coast of Nova Scotia, and offshore on Georges and Browns banks (Table 4; Fig. 3). Regional environmental factors however, could equally well be affecting larval morphological characters, such as size, as was found in juvenile and adult lobsters (Templeman 1936b). The two environmental variables that have been found to most affect the ultimate size of planktonic crustaceans are temperature and feeding conditions (Deevey 1960; Vidal 1980; Klein Breteler and Gonzalez 1982, 1988). It is well established that an optimum temperature exists for the development of maximum body size in larvae decapods (Rothlisberg 1979; Johns 1981; Anger 1984). MacKenzie (1985, 1988) found that there was a significant effect of temperature on the settling size of lobster, with stage V cultured at 15–18°C being larger than those reared at 10, 12, or 22°C. However, the effect of temperature on larval lobster body size was not realized for several moults in laboratory culture (MacKenzie 1988). The size discrepancy that we observed between inshore and offshore larvae, if at least partially caused by environmental temperature, must occur during the embryonic stage in the egg.

Once eggs are extruded onto the mother's swimmerets, the growth and development of the embryos must be controlled primarily by environmental temperature. Squires (1970) found no perceptable embryonic development in extruded eggs when bottom temperatures were below 3.5°C by observing egg-yolk content in Port-au-Port, Newfoundland, between December and April. Perkins (1972) observed that embryonic development ceased in some clutches and was barely discernable in others below 5–6°C in Boothbay Harbor, Maine. However, embryos of later clutches developed at faster rates than older clutches in the same thermal conditions, such that increases in eye index values were noted in younger clutches down to the 0.1–1.5°C range. Campbell (1986) estimated a thermal threshold of 3.4°C for his calculations of degree-days required for extrusion of eggs to hatching of larvae in the Bay of Fundy.

Standard carapace length plotted against calculated degree-days in bottom waters was best fitted by a linear regression using a thermal threshold of 3.5°C ($R^2 = 0.88$; Table 7) after Squires (1970) and Campbell (1986), although this was not much of an improvement over a 5°C threshold ($R^2 = 0.84$) after Perkins (1972). Browns and Georges bank lobsters are estimated to achieve maximum degree-days of 2221 and 2333, respectively, by migrating seasonally to the warmer waters in the offshore canyons. These values are similar to the 2350 degree-days required for lobster eggs to hatch while held at seasonal temperatures at Boothbay Harbor, Maine (Perkins 1972). The eggs hatched along the Atlantic coast of Nova Scotia appear to require the least number of degree-days, 1535–1647, whereas St. Georges Bay and Sydney Bight have intermediate requirements of 1683–1823 degree-days. It is possible that our inshore estimates of degree-days required for egg hatching are low because females may carry their eggs more than 11 mo or may move locally into warmer shallow water (Templeman 1935b; Munroe and Therriault 1983; Ennis 1984; Duggan and Pringle 1988).

TABLE 6. Relationship between selected morphological characteristics of stage I lobsters and the corresponding sea surface temperature (T) at the seven study sites (*$P < 0.05$, **$P < 0.01$).

Character	Regression equation	R^2
Standard carapace length	$Y = 2.22 - 0.02T$	0.87**
Maximum carapace length	$Y = 2.60 - 0.02T$	0.86**
Rostrum length	$Y = 2.09 - 0.01T$	0.44
Depth of second abdominal segment	$Y = 0.94 - 0.01T$	0.52
Length of fifth abdominal segment	$Y = 0.90 - 0.01T$	0.82**
Length of sixth abdominal segment	$Y = 0.89 - 0.02T$	0.69*
Depth of sixth abdominal segment	$Y = 0.58 - 0.01T$	0.88**

TABLE 7. Relationship between selected morphological characters of stage I lobsters and the calculated average annual degree-day bottom temperatures[a] above 3.5°C[b] (T) for the 11-mo period prior to hatching[c] at the seven study sites (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

Character	Regression equation	R^2
Standard carapace length	$Y = 1.79 + 1.0 \times 10^{-4}T$	0.88***
Maximum carapace length	$Y = 2.11 + 1.2 \times 10^{-4}T$	0.95***
Rostrum length	$Y = 1.75 + 9.1 \times 10^{-5}T$	0.55*
Depth of second abdominal segment	$Y = 0.63 + 7.3 \times 10^{-5}T$	0.50
Length of fifth abdominal segment	$Y = 0.70 + 4.6 \times 10^{-5}T$	0.69**
Length of sixth abdominal segment	$Y = 0.44 + 1.1 \times 10^{-4}T$	0.69*
Depth of sixth abdominal segment	$Y = 0.31 + 6.4 \times 10^{-5}T$	0.89***

[a]Calculated from Drinkwater and Trites (1987) and Petrie (1990).
[b]Temperature above which embryonic development occurs (Squires 1970; Campbell and Stasko 1986).
[c]Approximate duration of extruded eggs (Templeman 1940; Robichaud and Campbell 1991).

TABLE 8. DFA of the residuals of larval morphometric characters around the Maritimes after temperature effects on embryonic development have been removed. Functions included show a significant percentage ($P < 0.05$) of the variation.

Variable	Function 1	Function 2
Depth of second abdominal segment	0.838	0.276
Length of fifth abdominal segment	−0.580	0.973
Rostrum length	0.408	0.243
Length of sixth abdominal segment	−0.105	−0.352
Percent variation explained by function	78.5	17.9
Significance of function	0.00	0.01
Canonical correlation	0.50	0.26

The amount of energy or lipid reserve in the egg could also influence the size of larvae produced and this may be related to the nutritional state of the mother. Nothing is known about the variability of lobster egg yolk in nature. However, the dry egg weight of lobsters is known to be positively correlated with the size of the mother (Attard and Hudon 1987). Female lobsters, on average, trapped over the offshore banks are larger than those caught in the inshore fishery (Skud 1969; Campbell and Pezzack 1986; Pezzack 1987). It is possible to explain the larger offshore larvae if offshore lobsters extrude larger eggs with more energy storage.

It appears, then, that offshore lobster embryos may benefit from higher average temperatures and more energy reserves in the egg, both of which could help to explain the larger size attained at hatching. The morphometric differences described earlier between larval lobster populations along the Atlantic coast of Nova Scotia, southwest Nova Scotia, and the offshore banks disappeared once the effect of bottom temperatures, expressed as degree-days above 3.5°C, was factored out (Table 8; Fig. 4). St. Georges Bay and Sydney Bight remain separate on morphometric grounds which means that larval lobsters in the southern Gulf of St. Lawrence are genetically distinct and/or modified by maternal size, food resources, or a further unknown environmental factor.

A number of approaches other than morphometric criteria have been used to try to separate the lobster population into stocks or subpopulations for fisheries management purposes. Smaller immature lobsters, which predominate in inshore

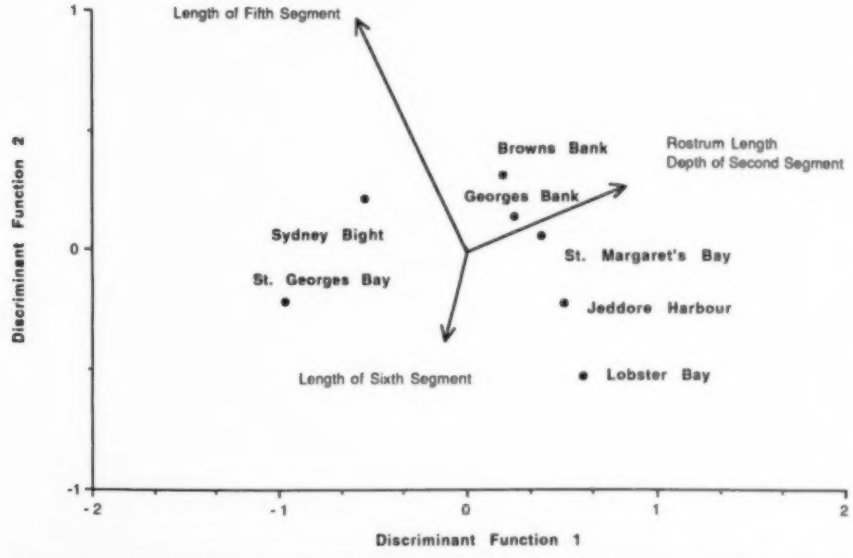

FIG. 4. DFA of lobster larvae morphometric variables calculated as residuals from the regressions of morphology against bottom water degree-days above 3.4°C in the preceding 11 mo.

TABLE 9. Separation of two regions in the Maritimes (percentage correct classification) with DFA of the residuals of the larval morphometeric characters after temperature effects have been removed.

Region	No. of larvae	Predicted group membership (%) Atlantic waters	Cape Breton inshore
Atlantic waters	254	74.8	25.2
Cape Breton inshore	113	25.7	74.3

waters, are relatively sedentary, moving less than 25 km seasonally (Wilder 1963; Fogarty et al. 1980; Krouse 1980; Ennis 1984; Campbell and Stasko 1986), whereas at least some of the mature larger lobsters off Grand Manan (Campbell 1986), Browns Bank, (Pezzack and Duggan 1986), Georges Bank (Uzmann et al. 1977), Rhode Island (Fogarty et al. 1980), and possibly off southwestern Nova Scotia (Campbell 1989a) are known to undertake longer annual deepwater migrations from summer depths of less than 100 m to greater than 200 m in the winter. Cape Sable Island, N.S., appears to be the dividing line between the presence and absence of these deepwater movements. West of Cape Sable, 19% of the lobsters tagged in inshore waters were recaptured in deep water whereas only 1.2% were so recaptured from releases to the east (Campbell 1989a, 1989b). This finding is supported by inshore release studies northeast along the Nova Scotia coast at Jeddore Harbour, Halifax Co., where one lobster of 2059 tagged and 799 recovered moved greater than 25 km (R. Duggan, Fisheries and Oceans, Halifax, N.S., pers. comm.) and at New Harbour, Guysborough County, where one tagged lobster of 465 recaptured had moved greater than 25 km, to Browns Bank (Miller et al. 1989). Results of the latter two studies are more similar to the inexplicable long-distance emigration of mature lobsters south from inshore regions around the Gulf of Maine (Krouse 1980; Campbell and Stasko 1986; Campbell 1989a). Tagging studies on lobsters in the deepwater basins of the Gulf of Maine indicate widespread movements in all directions in the Gulf (Anonymous 1985). Adult lobster behaviour, therefore, lends support to the results of our larval morphological approach, with extensive movements occurring within the Gulf of Maine and further south but with little or no exchange occurring with or between the Atlantic coast of Nova Scotia and the Gulf of St. Lawrence. It appears that there is ample opportunity for genetic exchange as a result of adult movements within the Gulf of Maine.

Larval lobsters have a planktonic period of 1–2 mo, depending on temperature and food conditions, which makes widespread passive displacement possible by coastal advective and dispersive processes (Harding and Trites 1988). A planktonic stage will increase gene flow and decrease local adaptation if this effect of dispersal is not minimized by reduced viability or fecundity (Strathmann 1985; Hedgecock 1986). Rogers et al. (1968), working off Rhode Island, found that stage I lobsters predominated offshore in the catches, while the fourth stages were most abundant closer to shore, which they inferred represents an inshore drift of larvae consistent with the surface circulation. Stasko (1978), likewise, proposed that larvae hatched on Browns Bank would drift towards the Nova Scotian coast to settle and that older lobsters could complete the ontogenic cycle by migrating offshore, ultimately to release young. Dadswell (1979) inferred from coastal surface circulation that both southwest Nova Scotia and the Magdalens were "closed cells" where lobster larvae would be retained. All other regions in the Maritimes were thought to lose an unspecified amount of their larval recruitment downstream in the prevailing current. Harding and Trites (1988, 1989) have since deduced from larval abundance, estimated larval duration, and drift bottle returns that offshore larvae from Browns Bank could be advected east of the Cape Sable region, west of Yarmouth, N.S., to the Bay of Fundy, and southwest along the coast of Maine as far as Casco Bay. However, the circulation in the Gulf of Maine is more complicated, due to the presence of numerous eddies, than indicated from drift bottle returns (see Brooks 1985). Lobster larvae, therefore, could be carried to distant locations, but it has not yet been possible to estimate the quantity or viability of these displaced larvae. We can only conclude that gene flow by larval lobster dispersal will be much greater within semi-enclosed circulation systems such as the Gulf of Maine and the southern Gulf of St. Lawrence than between these regions.

Physiological differences were observed in the environmental control of the timing of vitellogenesis and egg extrusion in lobsters collected from Martha's Vineyard, Massachusetts (Nelson et al. 1983), and Miminegash, P.E.I. (Aiken and Waddy 1986). The timing of egg extrusion by Massachusetts lobsters was thought to be regulated by seasonal progression from short to long daylength whereas the lobsters from the southern Gulf of St. Lawrence, maintained at natural temperatures, required no stimulation from daylength. Nelson (1986) proposed that this discrepancy could be under genetic control. Aiken and Waddy (1989) now feel that no physiological difference exists between these areas and that spawning time of inshore lobsters is synchronized by the seasonal temperature cycle, with photoperiod assuming a secondary role if the winter seawater temperature remains abnormally high.

Enzyme electrophoresis has been used to examine the genetic interrelations of lobsters collected from eight locations between the Gulf of St. Lawrence and the inshore and offshore regions off Massachusetts (Tracey et al. 1975). Hedgecock et al. (1975) presented evidence for Mendelian inheritance of protein variants in five of the eight most polymorphic loci. A total of 44 loci were studied in relation to 18 electrophoretically detectable proteins (Tracey et al. 1975). The average proportion of heterozygous loci was low at 3.8% but a total separation was found at the malic enzyme locus between inshore Massachusetts and Prince Edward Island (Tracey et al. 1975). The alternative alleles found in these two populations were present in approximately equal frequencies in offshore lobsters. Burton and Feldman (1982) and Shaklee (1983) have found differentiation at additional loci in the data set reported by Tracey et al. (1975). However, Shaklee (1983) concluded that the results of Tracey et al. (1975) are based on too few lobsters, weak enzyme activity, and other limitations, making them inconclusive.

Landing patterns have also been used in an attempt to distinguish lobster stocks. Fishing intensity is sufficiently high that lobster landings are generally thought to be a close approximation of the annual recruitment into the local population (see Robinson 1979; Campbell and Robinson 1983). Campbell and Mohn (1983) used principal component analysis together with cluster techniques to classify 32 lobster landing patterns in Canada (31 regions) and Maine. Similarly, Harding et al. (1983) examined annual lobster landings in 18 regions between Newfoundland and New York from 1928 to 1979 using correlation analysis correcting for autocorrelation between proximal years. In both approaches the Gulf of Maine and the Gulf of St. Lawrence were found to form discrete groups, but the Atlantic coast of Nova Scotia was considered by Campbell and Mohn (1983) to be a transitional group sufficiently distinct to merit a third

category. This minor difference between the two classifications, considering the differences in their approach, probably resulted from the former analysis taking into account adjacent regions only.

The results of the present morphological study, together with the current understanding of adult movements, larval dispersion, electrophoretically detectable enzymes, and landing patterns, all suggest that a difference exists between the lobster stocks of the Gulf of St. Lawrence and Gulf of Maine. These differences may be partly genetic in origin because of partial geographic and hydrographic isolation. However, one-way genetic exchanges have occurred since the turn of the century through extensive introduction of Gulf of St. Lawrence lobsters to the Gulf of Maine (Knight 1919; Chaisson 1932; Templeman 1944b; Robichaud 1990). It is possible also that the intense fishing pressure over the past century in inshore areas, particularly the southern Gulf of St. Lawrence, may be regionally altering the gene pool by selectively removing larger lobsters. Further understanding of the lobster population will come with more extensive genetic studies. DNA technology should be applied to understand this important issue.

Acknowledgements

We would like to thank Bob Miller for arranging the participation of Zhensui Zheng in this study. Susan Waddy provided us with advice on matters physiological. Julian Addison, Doug Pezzack, John Tremblay, and an anonymous referee made helpful suggestions for improving various drafts of this manuscript.

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Application of Empirical Size-Dependent Models of Larval Fish Vital Rates to the Study of Production: Accuracy and Association with Adult Stock Dynamics in a Comparison among Species

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Pepin, P. 1993. Application of empirical size-dependent models of larval fish vital rates to the study of production: accuracy and association with adult stock dynamics in a comparison among species. Can. J. Fish. Aquat. Sci. 50: 53–59.

A simple model of the size-dependent change in biomass of a cohort of fish larvae based on empirically derived relationships for growth and mortality is presented. Model predictions are compared with data for seven species of larval fish sampled on the Grand Banks of Newfoundland in 1980–81 to determine (1) whether the slope of the size-dependent larval biomass curve is significantly different from the general empirical model, and (2) whether the slope and intercept of the biomass curve are reflective of relative year-class strength and stock abundance, respectively. The slope of the size-dependent larval biomass curve is not significantly different from that predicted by the model in six of seven cases. The slope of the larval biomass curve is not significantly correlated with standardized year-class strength. The height of the curve (i.e. intercept) is only weakly associated with adult stock biomass. I conclude from this set of observations that estimates of larval fish vital rates may not be measured with sufficient accuracy to permit detection of a significant association between deviations from the general empirical model and the factors that may influence survivorship during the larval phase in a comparison across species.

Un modèle simple pour décrire le changement de la biomasse d'une cohorte de poissons à l'état larvaire, en fonction de la taille et selon des rapports dérivés empiriquement portant sur la croissance et la mortalité, est présenté dans cet article. Les prévisions du modèle sont comparées aux données portant sur des larves de poissons appartenant à sept espèces, échantillonnées sur les Grands Bancs de Terre-Neuve en 1980–1981. Ces comparaisons visent à déterminer 1) si la pente de la courbe décrivant la biomasse des larves en fonction de la taille diffère significativement du modèle empirique général et 2) si la pente et l'intersection à l'ordonnée de la courbe de la biomasse sont représentatives des effectifs relatifs d'une classe d'âge et de l'abondance d'un stock, respectivement. La pente de la courbe ne diffère pas significativement de celle prévue par le modèle dans six cas sur sept. Elle n'est pas en corrélation significative avec l'effectif standardisé des classes d'âge. La hauteur de la courbe (point d'intersection avec l'axe des ordonnées) n'est que faiblement associée à la biomasse du stock d'adultes. Cet ensemble d'observations amène l'auteur à conclure que les estimations de taux démographiques de larves de poissons ne peuvent pas être obtenus avec une précision telle qu'on pourrait détecter une assocation significative entre les écarts par rapport au modèle empirique général et les facteurs qui peuvent agir sur la survie au cours de la phase larvaire, lors d'une comparaison interspécifique.

Received March 24, 1992
Accepted July 14, 1992
(JB447)

Reçu le 24 mars 1992
Accepté le 14 juillet 1992

In the study of pelagic marine ecosystems, estimates of energy flow between trophic levels are based on the concept that organisms are part of a food web (see Ulanowicz and Platt 1985). The degree of complexity with which authors have viewed interspecific interactions in marine ecosystems varies greatly. For example, Hardy (1924) proposed a complex gamut of species interactions through the life cycle of North Sea herring. Alternatively, some authors (e.g. Silvert and Platt 1978, 1980; Borgmann 1983; Dickie et al. 1987) have reduced ecosystems to size-dependent interactions in which the flow of energy through the food web is described by allometric models. Although the latter approach may represent an oversimplification, a simple size-dependent model of trophic interactions provides a framework which has been used effectively to study the distribution of biomass in pelagic ecosystems throughout the world (Sheldon et al. 1972, 1977). General empirical models have also been used to estimate species- or taxa-specific production features (e.g. Borgmann 1983; Huntley and Boyd 1984; McClatchie 1987; Morin et al. 1988).

In an attempt to synthesize the information available on the vital rates of fish larvae, Houde (1989) and Pepin (1991) estimated empirical relationships that describe daily growth and mortality rates of larval fish as functions of size and environmental temperatures. Miller et al. (1988), Beyer (1989), Houde (1989), and Pepin (1991) suggested that such relationships can form the basis for studies linking early life history survival with recruitment variability. Deviations from general models may be linked with ecological or environmental features. However, to compare independent observations with general empirical relationships, it is essential that we be able to detect departures frm these models. Before this can be accomplished reliably, it is necessary to ask whether estimates of larval fish abundance are sufficiently accurate to detect variations in survivorship during the early life history.

To answer this question, I present a simple model of the change in biomass of a developing cohort of larval fish and I contrast the predictions with observations from several species from a large ecosystem, the Grand Banks of Newfoundland.

The model is based on a general review of vital rates (Pepin 1991). The field data are derived from surveys of the Grand Banks conducted during the period 1980–81 (Bonnyman 1981; Conover 1981; Hollibaugh 1981; Strong 1981). The objectives are to determine (1) whether the slopes of the size-dependent larval biomass curve for several species on the Grand Banks are significantly different from that predicted from the general empirical model and (2) whether the slope and intercept of the size-dependent biomass curve can be contrasted across species to determine whether these statistics are reflective of year-class strength (relative to each species' long-term mean) and/or stock abundance.

Model

The size distribution of organisms in the pelagic food web can be described by a time-dependent equation which assumes that the rate of energy transfer is dependent principally on particle growth and predation (Silvert and Platt 1978) such that

$$(1)\quad \frac{\partial \beta}{\partial t} + \frac{\partial}{\partial w}(\beta G) + M\beta = 0$$

where $\beta(w,t)$ is the biomass density function such that $b(w,t) = \beta(w,t)dw$ is the mass of particles per unit volume of water, in the interval of sizes w and $w + dw$ at time t. The specific growth rate of particles is $G(w)$ (i.e. $dw/dt = G(w)\cdot w = g(w)$) and the specific rate of change in the number of particles is $M(w)$ (i.e. $dN/dt = -M(w)N$ where $N = \beta/w$). Equation 1 assumes that reproduction within the size distribution is negligible. Silvert and Platt (1978) gave the steady-state solution to equation 1 (i.e. $\partial\beta/\partial t = 0$) as

$$(2)\quad \beta(w) = \tau(w)e^{-\int M(w')\tau(w')dw'}$$

where $\tau(w) \equiv 1/g(w)$ is the turnover rate of particles of size w (i.e. the time required to grow through size w).

Elements of equation 2 were obtained from Pepin's (1991) empirical summary of the vital rates of fish larvae in relation to animal size and environmental temperature. The growth (millimetres per day) of feeding larvae was described as

$$(3)\quad g[l,T] = 0.031e^{0.073T}l^{0.54}$$

where l is the length (millimetres) of the individual and T is the environmental temperature (degrees Celsius). The instantaneous mortality rate (per day) was described as

$$(4)\quad M[l,T] = 0.25e^{0.067T}l^{-0.68}.$$

The standard error associated with the size (l) parameters for growth (equation 3) and mortality (equation 4) was 0.18 and 0.23, respectively (Pepin 1991). Because the parameters for the effect of temperature on growth and mortality are approximately equal, the net effect of temperature on the development of biomass is not significantly different from 0. Therefore, I do not include this term in the model's parameterization.

Inserting equations 3 and 4 into equation 2 yields

$$(5)\quad \beta(l) = l^{-0.54}e^{al^{-0.21}}.$$

If we assume that $w \propto l^4$ (Laurence 1978; Pepin, unpubl. data), equation 5 gives

$$(6)\quad \beta(w) = w^{-0.14}e^{\alpha w^{-0.05}}$$

where α is a constant. If we integrate the biomass over a logarithmic size interval, $b(w) = w\cdot\beta(w)$, we obtain

$$(7)\quad b(w) = w^{0.86}e^{\alpha w^{-0.05}}.$$

Relative to the first term on the right-hand side of equation 7 ($w^{0.86}$), the exponentiated term can be considered to be approximately constant with changing size (w). Equation 7 states that the biomass of a cohort of fish larvae can be considered to be proportional to $w^{0.86}$. The standard error of the exponent of weight is 0.10, after transformation, and is approximated as the sum of the standard errors of the growth and mortality components. This value is similar to that obtained by Pepin (1991) for the general empirical model of larval survivorship.

Data Sources and Analysis

Ichthyoplankton Abundance

The abundance of fish larvae on the Grand Banks was determined from a series of 13 cruises during the period from March to November 1980. Details of the methods are given in Bonnyman (1981), Hollibaugh (1981), and Strong (1981). Seven surveys included up to 50 sampling stations on four east–west transects (44°N, 45°N, 46°N, 47°N) along which stations were spaced approximately 20 nautical miles apart. Six surveys included between 27 and 59 sampling stations located along 13 fixed transects that crossed the periphery of the Grand Banks (Hollibaugh 1981). Sampling was conducted using 63-cm bongo nets equipped with 333-μm-mesh nets and flowmeters. Nets were towed obliquely to a depth of approximately 60–70 m at a ship speed of 2 knots (Bonnyman 1981; Strong 1981). Samples were preserved in 5% formalin. Fifty-nine to 100% of samples from individual cruises were analyzed for fish larvae (Bonnyman 1981). All fish larvae were sorted from the samples, identified to the lowest taxonomic order possible, and measured for standard length to the nearest 0.1 mm. Ichthyoplankton numbers for each 1-mm length class were converted to abundance (numbers per cubic metre) based on the volume of water filtered. The length frequency distribution for each species and cruise was estimated by assuming that all stations represented equal areas and that sampling was representative of each population's distribution. The cumulative length frequency distribution was produced by summing the length frequency distribution over all cruises and correcting for the proportion of samples sorted. Length was converted to weight using estimated length–weight relationships (P. Pepin, unpubl. data). Biomass (grams dry weight per cubic metre) was estimated by multiplying numbers in each size interval by the mean weight for the interval. This yielded the larval production curve of biomass in relation to weight. The biomass of each weight interval was taken as the total production during the spawning season for populations residing on the Grand Banks. Measurement errors due to sampling variability and patchiness were not determined.

Seven species or species groups were present in sufficient numbers to estimate size-dependent biomass curves. These are American plaice (*Hippoglossoides platessoides*), capelin (*Mallotus villosus*), Atlantic cod (*Gadus morhua*), redfish (*Sebastes* sp.), sand lance (*Ammodytes* sp.), witch flounder (*Glyptocephalus cynoglossus*), and yellowtail flounder (*Pleuronectes ferrugineus*, previously *Limanda ferruginea*).

Year-Class Strength and Adult Stock Biomass

Data on year-class strength and adult stock biomass were obtained from population assessments prepared for the Northwest Atlantic Fisheries Organization (NAFO). Details about sources, assessment method, stock region, relative year-class strength, and stock abundance are listed in Table 1.

To contrast year-class strength across species, it is not possible to use absolute measures of year-class strength because the recruitment estimates for the different species are calculated using different methods and at different ages. Furthermore, the second objective of this study is to assess whether deviations from the general empirical model of larval production (equation 7) are associated with fluctuations in recruitment relative to each species' long-term variability. Estimates of year-class strength for each species were standardized relative to the standard deviation of the time series for that species or stock such that

$$(8) \quad \mathcal{R}_i = \frac{\log R_{i,80} - \overline{\log R_i}}{s_{i,\log R}}$$

where $\mathcal{R}_i$ is the standardized recruitment index for species i, $R_{i,80}$ is the recruitment of the 1980 year-class for species i, $\overline{\log R_i}$ is the mean recruitment from the time series for each species, and s is the sample standard deviation, defined by

$$(9) \quad s^2 = \frac{1}{N}\sum_{i=1}^{N}(X_i - \bar{X})^2$$

where N is the number of years in the recruitment time series and X_i is the measure of recruitment (i.e. $\log R_i$). $\mathcal{R}_i$ provides a measure of year-class success relative to each species' natural variability and thus permits a comparison of relative recruitment between species. $\mathcal{R}_i$ also encompasses mesurement error associated with the assessment of populations, but I assume that the effect of this factor is constant across species. Adult stock biomass represents the abundance of mature fish.

Analysis

The slope and intercept of the size-dependent larval biomass curves were determined using linear regression on the transformed data

$$(10) \quad \log(b(w)) = a + c\log(w)$$

to estimate the rate of change in biomass ($b(w)$: grams dry weight per cubic metre) in relation to weight (w: micrograms dry weight). The terms a and c represent the intercept and slope of the relationship. The regression was not weighted by the number of samples used to obtain the biomass of each size category. The slope (c) was compared with the theoretical predictions (equation 7) using a two-tailed t-test. Because the model is based on empirical relationships, the standard error associated with these relationships is incorporated into the analysis. The standard error of the theoretical model (equation 7) is 0.10. The slope of equation 10 is assumed to be representative of the size-dependent production of fish larvae. The intercept is assumed to be representative of the initial biomass of fish larvae. Although this term is not explicitly indicated in equation 7, it is implicit through our knowledge of population biology and energy conservation in ecosystems. From a system's perspective, such a term would reflect nutrient input. From a population's perspective, such a term would reflect the initial biomass of larvae.

Comparison of the slopes with relative year-class strength across species was done using Pearson's product-moment correlation coefficient (r) as well as Kendall's τ, a nonparametric measure of association. The intercept with adult stock biomass was compared in the same manner. The hypothesis being tested in both comparisons is that there should be a positive association between relative year-class strength (adult stock biomass) and the slope (intercept) of the larval biomass function. Both parametric and nonparametric measures of correlation are used because the former measures deviations from a linear relationship between two variables whereas the latter measures whether the association has a monotonic relationship. Because I have no reason to assume that either measure of association is a better reflection of the type of relationship between the variables considered, I choose to present the results from both methods of analysis.

Results

The size-dependent biomass function of the seven species sampled in the 1980 survey all show a significant ($p < 0.05$) increase with increasing size of larvae (Fig. 1) which is con-

TABLE 1. Summary of information on year-class strength and stock abundance for the seven species used in the study. Assessment methods include sequential population analysis (SPA), research vessel surveys (RV), and hydroacoustics surveys (HAS). Age refers to the age at which recruitment is measured, N is the number of observations in the recruitment time series for that species which were used in the calculation of the long-term variability (s), year-class strength is standardized relative to the standard deviation of the recruitment time series (s), and adult biomass is measured in thousands of tonnes. Data for stock biomass of witch flounder and redfish represent the average biomass during the early 1980s. Stock biomass for capelin was estimated by back-calculating the 1980 biomass from the 1981 survey using average annual rates of change in numbers and biomass. N/A indicates that information for 1980 was not available. Personal communications are from the scientific staff of the Northwest Atlantic Fisheries Center, St. John's, Newfoundland, Canada.

Species	Stock	Assessment method	Age (yr)	N	Year-class strength	Adult biomass	Source(s)
American plaice (*Hippoglossoides platessoides*)	3LNO	SPA	6	21	−0.004	324	Brodie and Baird 1991
Capelin (*Mallotus villosus*)	3LNO	HAS	3 + 4	9	−0.397	303	J. Carscadden, pers. comm.
Atlantic cod (*Gadus morhua*)	3NO	SPA	3	29	−0.532	131	Baird and Bishop 1988
Redfish sp. (*Sebastes* sp.)	3NO	RV	1 + 2	9	0.872	140	Power and Baird 1991; Myers et al. 1990
Sand lance (*Ammodytes* sp.)	N/A	N/A	N/A	—	N/A	N/A	
Witch flounder (*Glyptocephalus cynoglossus*)	3LNO	RV	N/A	—	N/A	10–30	W. Brodie, pers. comm.
Yellowtail flounder (*Pleuronectes ferruginens*)	3LNO	SPA	5	18	−0.389	38–54	Brodie 1986

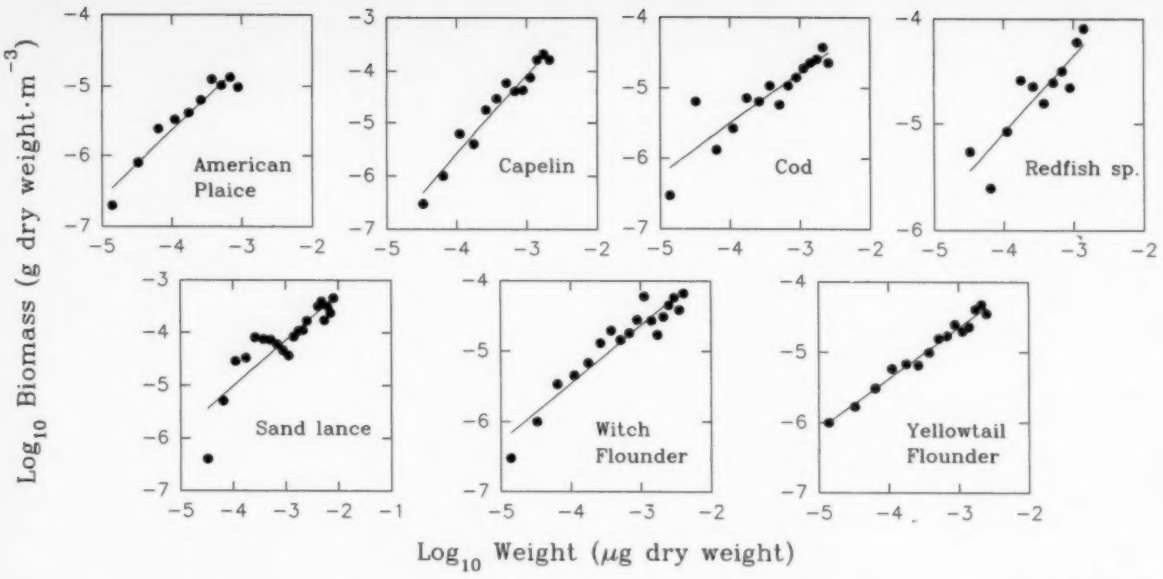

FIG. 1. Logarithm of larval fish biomass plotted in relation to the logarithm of larval fish weight for the seven species used in this study. The slope and intercept of the larval biomass curves are listed in Table 2.

TABLE 2. Regression coeffients, r^2, and the t-value for the difference between observed and predicted (equation 7) slopes of the size-dependent larval biomass curves. Figures in parentheses represent the standard error for each regression coefficient. Symbols next to the t-value indicate the significance level (ns, not significant; $*p < 0.05$).

Species	Intercept	Slope	r^2	t-value
American plaice	−1.82 (0.36)	0.95 (0.095)	0.93	0.65 ns
Capelin	−0.11 (0.28)	1.35 (0.090)	0.93	3.64 *
Atlantic cod	−2.59 (0.34)	0.73 (0.097)	0.81	0.93 ns
Redfish sp.	−2.13 (0.46)	0.74 (0.13)	0.78	0.73 ns
Sand lance	−0.71 (0.71)	1.08 (0.23)	0.52	0.88 ns
Witch flounder	−2.15 (0.23)	0.83 (0.070)	0.90	0.25 ns
Yellowtail flounder	−2.48 (0.11)	0.72 (0.031)	0.98	1.33 ns

sistent with the model's (equation 7) general prediction. The slope and intercepts of the size-dependent biomass relationships show some variability, and the accuracy of the parameters appears to be weak (Table 2). Overall, larvae of pelagic forage fish are more abundant than larvae of either flatfish or groundfish. The abundance of species in the latter two groups is about equal in terms of biomass.

Although the species-specific values of the slopes of the biomass functions (Fig. 1; Table 2) range from 0.72 to 1.35, the slope of the curve is significantly different from the theoretical prediction (equation 7) in only one instance (capelin; Table 2). The slope of the size-dependent larval biomass curve for sand lance is greater than the predicted slope but the high standard error renders the result nonsignificant. The slope of the larval biomass curve for all demersal species is close to the theoretical value of 0.86.

The slope of the larval biomass curves was not correlated with the standardized recruitment ($\mathcal{R}$) indices for the five species for which data were available (Fig. 2). The degree of association is low ($r = -0.26$, $p > 0.2$, $\tau = -0.10$, $p > 0.2$, $n = 5$). This is a clear indication that the general size-dependent larval production model is not adequate for comparison of potential year-class strength between species based on estimated larval fish abundance.

The height of the larval biomass curves (i.e. the intercept) shows some association with fishable stock biomass (Fig. 3) ($r = 0.69$, $p = 0.12$, $\tau = 0.47$, $p = 0.19$, $n = 6$). However, Fig. 3 shows that this statistical association is only a reflection of the general trend. There is considerable scatter about the mean relationship suggesting that factors other than stock abundance determine relative larval production among species.

Discussion

Comparison of the pattern of size-dependent biomass production for seven species of fish larvae from the Grand Banks with the predictions from the general empirical model shows that under most circumstances, it is not possible to detect significant differences between model predictions and field observations despite substantial variation in the slope of the curve ($|\overline{\Delta c}| = 0.17$, $s_{\Delta c} = 0.15$). This evidence contrasts with one's expectation of a high r^2 for the larval biomass function (equations 7 and 10), since it represents an estimate of the relation-

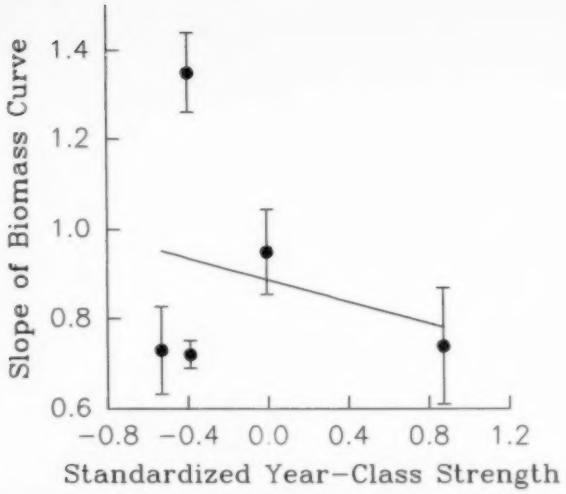

FIG. 2. Slope of the larval biomass curve plotted in relation to the standardized year-class strength of five species of fish from the Grand Banks of Newfoundland. The error bars represent the standard error of the slope of the size-dependent larval biomass curve. The regression line is presented only to provide a perspective against which to compare the distribution of points relative to the trend indicated by the correlation analyses.

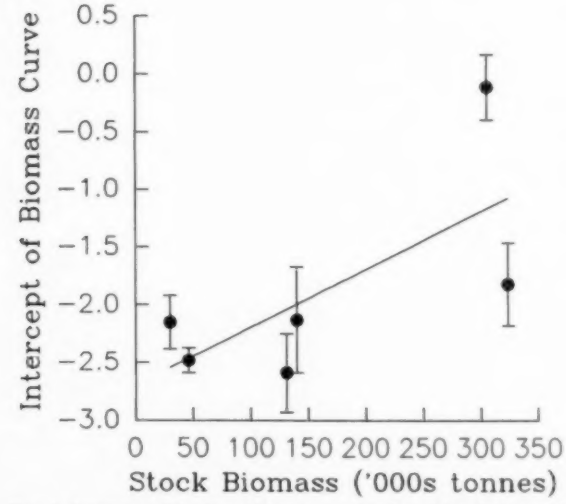

FIG. 3. Intercept of the larval biomass curve plotted in relation to the adult stock biomass for six species of fish from the Grand Banks of Newfoundland. The error bars represent the standard error of the slope of the size-dependent larval biomass curve. The regression line is presented only to provide a perspective against which to compare the distribution of points relative to the trend indicated by the correlation anlyses.

ship of wN versus w. It suggests that estimates of larval fish abundance are not sufficiently accurate to detect variations in survival which depart from an overall mean. The consistency in the pattern of larval biomass function between species indicates that general empirical models of larval fish rates may be appropriate forecasters of the general trend. However, failure to detect major departures from the model (up to a 25% variation from the predicted slope (equation 7) (Table 2) suggests that the application of size-dependent relationships to study fluctuations in general species-specific production characteristics (i.e. deviations from the mean) may not yield satisfactory results.

One must be cautious in generalizing the results of a single multispecies survey during one year and in a specific ecosystem to specific studies of year-class variations for a single stock. A potential limitation of the analysis presented above may be the attempt to contrast species on the basis of a general empirical model rather than focusing on differences between year-classes within a species. Each species might have average size-dependent growth and mortality functions that depart significantly from the general relationships estimated using information from a suite of species and studies (McGurk 1986; Houde 1989; Pepin 1991). This would affect our ability to associate departures in survivorship from the general model (equation 7) with fluctuations in year-class strength. Furthermore, different species may allocate a different proportion of their biomass to reproduction. This would affect our ability to associate larval production with stock size. Despite the potential modulators that could influence the usefulness of the general model (equation 7) for species-specific studies, the results of this study suggest that attempts to link larval fish dynamics to adult stock fluctuations are unlikely to produce results that are significantly different from the average functional relationship. Pepin (1991) found that when taxonomic deviations from the average functional relationships for growth and mortality were considered together, the net effect on survivorship was negligible. Furthermore, in the example presented in this study, it is evident that the estimates of larval fish abundance are not sufficiently accurate to allow detection of a significant departure from a model which represents the average of 28 species (clupeids, scrombrids, gadoids, pleuronectiformes) which come from a variety of environments (estuaries, shallow seas and banks, upwelling systems) and different types of surveys (Pepin 1991). The average larval production curve represents the change in biomass of cohorts required to maintain reproductive success of the adult populations. One must question whether species- or stock-specific surveys would produce estimates of vital rates that are sufficiently accurate to detect variations in year-class strength within a population.

Most estimates of larval fish vital rates are based on broad-scale population surveys (e.g. Zweifel and Smith 1981; Morse 1989). Although there is correspondence between environmental factors and mortality rates over large scales (e.g. Pepin 1987; Butler 1991), there is some doubt as to their association at the scale that is likely to influence variations in recruitment (Sissenwine 1984; Walters and Collie 1988; Peterman et al. 1988; this study). The average difference between minimum and maximum cumulative mortality (log(recruits/eggs)) for the 12 stocks studied by Garrod (1982) is 2.5 (range 0.94–4.6). this implies that the maximum survivorship to age 1 (N_1/N_0) for commercial fish stocks is 12.2 times the minimum survivorship (range 2.5–1000 times). On average one individual in 160 000 eggs spawned reaches the age of 1. The range of fluctuations in survivorship is 1 : 40 000 – 1 : 600 000. Smith (1985), Houde (1987), and Beyer (1989) have shown that by using average vital rates of larval fish, subtle variations in instantaneous development or mortality rates ($\approx \pm 5$–10%) can lead to fluctuations in survival that are sufficient to result in the general range of year-class strength observed for most commercial fish stocks (i.e. up to 100-fold variations). If we could simply fore-

cast when year-classes are likely to be extremely good or bad, then a significant advancement in management practices might be available. However, the results of this study suggest that it is doubtful whether the deviations in vital rates required to produce such variations in recruitment could be detected by the methods currently used. Given that most larval fish mortality rates can only be estimated to an accuracy of $\pm 10\%$ (Pepin 1991 and unpubl. data), attempts at refining methods that provide overall population average mortality rates are unlikely to provide useful information about survivorship. Survey methods have to be developed in such a manner that variations in the spatial and temporal patterns in survival can be detected if any link between recruitment variability and early life history dynamics is to be established. Until such time, egg and larval surveys may be best suited to estimating spawning stock size (e.g. Lo 1985; Hausser and Sissenwine 1991) rather than assessing whether there is an association between early life history survivorship and recruitment fluctuations.

In addition to measurement error associated with the size-dependent larval biomass function, there is also measurement error associated with recruitment estimates. Walter and Ludwig (1981) presented examples of the potential impact which measurement error may have on estimates of stock–recruitment relationships. It is evident from their work that the impact can be substantial. One must be careful in assigning the lack of correspondence between estimates of larval production and recruitment to poor estimates of larval fish abundance alone. General lack of accuracy in both components of that relationship can clearly influence our perspective of functional studies of early life history dynamics and recruitment variability. Methods to achieve greater reliability in estimates of abundance of different stages in the life history of fish need to be further developed in order to properly assess the period during which relative year-class strength is determined.

Allometric relationships have been derived for a wide variety of parameters with the objective of using them as potential tools in estimating ecological flows in ecosystems or predicting patterns of biomass distribution between trophic levels (Sheldon et al. 1977; Silvert and Platt 1980; Borgmann 1983; Peters 1983; Cousins 1985; Hargrave et al. 1985; Borgmann 1987; Dickie et al. 1987). Models derived from such observations have proven useful in making predictions at the systems level (Sheldon et al. 1977; Leach et al. 1987; Minns et al. 1987), but their application to a narrow range of body size or to a specific taxonomic group has been restricted because of species-specific differences in production characteristics and becasue of the difficulty in estimating confidence intervals around the predictions (e.g. Huntley and Boyd 1984; Morin et al. 1988). For example, Silvert and Platt (1980) proposed that the biomass of pelagic organisms in aquatic ecosystems should decrease in proportion to $W^{-0.22}$. However, Ware (1975) proposed that the biomass of a cohort of fish larvae must increase to compensate for mortality during the egg stage; otherwise, recruitment would never provide replacement for the spawning population. The data for the Grand Banks show that larval fish exhibit a pattern consistent with Ware's (1975) predictions for the general trend in biomass through time, at least until they attain a weight of approximately 6 mg dry weight ($\approx$20 mm). There is considerable variability in the relationship for individual species, and very little of that variability can be measured with sufficient accuracy to detect departures from the mean functional model of the system. This is an indication that despite general consistency of patterns in marine ecosystems (e.g. Sheldon et al. 1972, 1977), the dynamics of individual species or taxa can exhibit substantial deviations from the average system characteristics without being statistically measurable. Ecosystem generalities may not be applicable to individual species (Peterson and Wroblewski 1984). Similarly, average functional relationships for growth, mortality, and survivorship of larval fish may only be useful in describing general trends in the abundance of organisms and should not form the basis for assessing short-term (e.g. annual) deviations from the mean. This conclusion is in contrast with Borgmann and Whittle's (1983) proposal that general allometric models could be used to detect variations in ecosystem characteristics. Such a contrast in model applicability can be due to the greater accuracy associated with general estimates of the distribution of biomass in pelagic systems relative to the accuracy of estimates of abundance for individual species. Laval fish abundance is generally less than $1 \cdot m^{-3}$ (Smith and Richardson 1977). Detecting subtle changes in the abundance of larval fish is difficult, particularly as the larvae become larger. Increasing avoidance capability, low abundance, and increasing aggregation associated with increasing size of larval fish can make estimates of abundance inaccurate. This creates problems in comparing model predictions with field observations becuse of the low accuracy associated with estimates of abundance. Upon contrasting several species with lengthy time series, Pepin and Myers (1991) found evidence that the length (duration) of the larval phase was significantly associated with the long-term variability in recruitment. The link between early life history variations and population dynamics may be more easily determined over the long term when a better perspective of the "average conditions" may be obtained.

Increasing the accuracy for estimates of larval fish vital rates may only be possible through more detailed knowledge of variations in both age and size. Many estimates of vital rates use the size frequency distribution as the basis for calculations, but the average size frequency distribution may not be an adequate proxy of physiological age which is needed for such purposes. Considerable variation in size for larvae of a given age exists (e.g. Penney and Evans 1985; Chambers et al. 1988) which can limit the accuracy of methods that fail to take this variation into account. A more meaningful representation of growth or mortality should be obtained by aging larvae using otolith microstructure. The extensive labour and cost involved in aging a sufficient number of larvae to obtain a representative sample of the population's age–length distribution have restricted the use of such approaches in the past. However, it is important to try to reduce measurement error of vital rates as much as possible. Future surveys need to obtain an accurate age–length key for fish larvae in order to link prerecruit features with population dynamics.

Despite the general consistency between the observed slope of the larval production curves and the theoretical prediction, the model (equation 7) has shown itself to be a poor predictive tool to contrast species in the same ecosystem. One may conclude that our current level of knowledge pertaining to the general features of larval fish vital rates (across species) is sufficient to understand general features of production for this taxonomic group (i.e. the slope is about right). However, in order to link events occurring during the larval stage with recruitment variability research, we must seek to make observations that allow us to quantify the events on the time and space scales that are relevant to the variations experienced by the larval population. In addition, there is a strong need for improved estimates of larval fish abundance which might be

obtained by increasing the volume of water sampled (e.g. larger sampling gear).

Acknowledgements

I wish to thank W. Brodie and J. Carscadden for their help in providing information on the flatfish and capelin stocks, respectively. I also thank P. T. P. Tsui of Mobil Oil Canada for granting permission to use data from the Grand Banks Oceanographic Studies. P. Shelton, M. Paranjape, T. Miller, and an anonymous reviewer provided valuable comments on this study.

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Cell-mediated Immune Response of Rainbow Trout (*Oncorhynchus mykiss*) to Larval *Pseudoterranova decipiens* (Nematoda; Ascaridoidea) following Sensitization to Live Sealworm, Sealworm Extract, and Nonhomologous Extracts

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Ramakrishna, N. R., M. D. B. Burt, and B. M. MacKinnon. 1993. Cell-mediated immune response of rainbow trout (*Oncorhynchus mykiss*) to larval *Pseudoterranova decipiens* (Nematoda; Ascaridoidea) following sensitization to live sealworm, sealworm extract, and nonhomologous extracts. Can. J. Fish. Aquat. Sci. 50: 60–65.

The delayed-type hypersensitivity (DTH) reaction (an in vivo manifestation of cell-mediated immunity) was studied in rainbow trout (*Oncorhynchus mykiss*) exposed to sealworm *Pseudoterranova decipiens* infections. Test fish, immunized with sealworm extract and live intact worms, were compared with control fish which received either phosphate-buffered saline (negative control) or nonhomologous cestode or nematode antigens (positive control). Test fish immunized with sealworm extract produced a typical DTH reaction to live sealworm challenge. The DTH reaction was similar to that in mammals and showed lymphoid and mononuclear cell infiltration. The electron microscopical studies revealed the presence of activated macrophages and plasma cells in the reaction zone. The specific response, following immunization with homologous antigens, supports the existence of T-cell function with anamnesis in rainbow trout exposed to sealworm antigens. However, when the test fish were immunized with live sealworms and later challenged with live sealworms, the secondary response was poor and showed no difference from controls and naive fish.

La réaction d'hypersensibilité retardée (une manifestation in vivo de l'immunité cellulaire) a été étudiée chez la truite arc-en-ciel (*Oncorhynchus mykiss*) exposée au ver du phoque (*Pseudoterranova decipiens*). Des sujets d'expérience, immunisés à partir d'extraits et de vers du phoque vivants et intacts, ont été comparés à des témoins qui avaient reçu une solution saline de tampon phosphate (contrôle négatif) ou des antigènes non-homologues de cestodes ou de nématodes (contrôle positif). Les sujets d'expérience immunisés avec des extraits du ver du phoque produisaient une réaction caractéristique d'hypersensibilité retardée lorsque leur organisme était challengé par des vers du phoque vivant. La réaction obtenue était semblable à celle observée chez les mammifères et on observait une infiltration des lymphocytes et des monocytes. Les examens faits au microscope électronique ont montré la présence de plasmocytes et de macrophages actifs dans la zone où se produisait la réaction. La réponse spécifique, par suite de l'immunisation avec les antigènes homologues, vient étayer l'existence de la fonction des lymphocytes T avec mémoire immunologique chez la truite arc-en-ciel exposée aux antigènes du ver du phoque. Cependant, lorsque les sujets d'expérience étaient immunisés avec des vers du phoque vivants et challengés avec des vers du phoque vivants, la réponse secondaire était mauvaise et il n'y avait pas de différence entre les contrôles et les sujets non exposés.

Received March 16, 1992
Accepted August 4, 1992
(JB436)

Reçu le 16 mars 1992
Accepté le 4 août 1992

The capacity of fish to mount an immune response to recognizably foreign material has been known since the beginning of this century. It is only in the last two decades that much interest has been shown in fish immunology both from a comparative (phylogenetic) and a practical (vaccination) point of view. Lengthy reviews of fish immunology have been published. These include reviews of immunization against various disease organisms (Snieszko 1970; Anderson 1974; Ellis 1988) and reviews of basic studies of immune responses and immune capabilities (Cushing 1970; Anderson 1974; Corbel 1975; Walczak 1985).

Although the fish immune system is not as elaborate or as sophisticated as the mammalian system (Ellis 1982), fish do possess nonspecific and specific defence mechanisms. As the lymphoid system is immature at hatching (Ellis 1977; Grace and Manning 1980; Botham and Manning 1981; van Loon et al. 1981), nonspecific defence mechanisms play an important role in the protection of early stages of fish (Ingram 1980; Ellis 1981; Fletcher 1982), while the specific humoral and cellular immune system enables the fish to survive hostile environments and maintain homeostasis in later stages of the life cycle.

Although considerable information is available on the fundamental principles of fish immunology, knowledge of immunological relationships between fish and their parasites is limited in comparison with that concerning fish and either bacteria or viruses (Houghton et al. 1988). Reviews on early immunological studies of the fish immune response to their parasite fauna have been made by several workers (Lom 1969; Snieszko 1969; Kennedy 1977; van Muiswinkel and Jagt 1984; Woo 1987). Recently, considerable progress has been made in investigating immunological relationships between host and parasite and in the development of vaccines, particularly in relation to

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the more commercially important protozoan and helminth diseases. Ichthyophthiriasis, costiasis, trypanosomiasis, and diplostomiasis have received specific attention by several researchers, as summarized by Houghton et al. (1988), with more recent reports by Thomas and Woo (1989, 1990a, 1990b) and Li and Woo (1991). Although some work has been reported on cestodes (e.g. Cottrell 1977; Sharp et al. 1989), copepods (e.g. Woo and Shariff 1990), and acanthocephala (Harris 1972), immunological studies on fish nematodes are rare.

In mammals and birds, cell-mediated immune responses have been considered to be the main defence against parasites (Cohen and Warren 1982). Little, however, is known about cell-mediated immunity (CMI) in fish against pathogens (Dorson 1984; Trust 1986; Jurd 1987), despite the well-documented occurrence of functional T lymphocytes and the known existence of a range of lymphokines (Ellis 1986; Graham and Secombes 1988). Little attention has been directed at understanding the cell-mediated immune responses in fish, which are manifested in graft rejection (Rijkers 1982), graft-versus-host reaction (Wright and Cooper 1982), mixed lymphocyte reaction (Miller et al. 1986), delayed-type hypersensitivity (DTH) reaction (Bartos and Sommer 1981), and macrophage migration inhibition (Jayaraman et al. 1979; Blazer et al. 1984; Blazer and Wolke 1984a, 1984b).

Pseudoterranova decipiens can naturally infect Atlantic salmon (*Salmo salar*) (pers. obs.). In view of the threat to the aquaculture industry posed by larval *P. decipiens* establishing themselves in the muscle of caged Atlantic salmon, the development of a vaccine preventing this could be greatly beneficial. Since there are no basic studies on the immunological relationships between fish hosts and sealworm, such studies are needed to assess whether a vaccine could protect fish against sealworm infections. Therefore, the objective of the present study was to obtain some basic immunological data regarding the "specificity" and "memory" of the host immune system in response to reinfection with sealworm. In the present study, the in vivo cell-mediated immune response was studied using rainbow trout (*Oncorhynchus mykiss*). Rainbow trout are easier to maintain than Atlantic salmon, being more readily adaptable to experimental manipulation of temperature, handling, and infection. The physiology of these trout is similar, however, to salmon, and thus these fish were used in the present experiment, with future experiments planned for Atlantic salmon.

Materials and Methods

Maintenance of Fish

One hundred rainbow trout (17–25 cm), 20 in each of five fish tanks, were acclimated to full-strength seawater (36 ppt) using "Instant Ocean" artificial sea salts in a recirculation system (Landless 1976). The fish were maintained in a temperature- and photoperiod-controlled room (15°C; 12 h light, 12 h dark) and were fed daily an appropriate grade of commercial trout feed.

Parasites

All sealworm larvae (L_3) used for live infections and antigenic preparation were collected from naturally infected Atlantic cod (*Gadus morhua*). The cestode *Monoecocestus americanus* and the pinworm *Welcomia evoluta* were collected live for nonhomologous antigen preparation from the gut of an infected porcupine (*Erethizon dorsatum*). All the parasites were rinsed several times in distilled water and physiological saline (0.85% NaCl) and then in sterile phosphate-buffered saline (PBS) (sodium chloride, 7.2 g; sodium phosphate (dibasic), 1.48 g; potassium phosphate (monobasic), 0.43 g; adjusted to pH 7.2) until free of visible debris and frozen at −70°C until the start of the experiment approximately 2 wk later.

Preparation of Antigenic Extract

The antigenic preparation was made by homogenising the parasites in PBS (pH 7.2) using a tissue grinder and then sonicating on ice for six 3-min periods with 30-s intervals. The protein concentration of the antigenic extract was determined using a BIORAD protein assay kit (Bradford 1976). The protein concentration of all three parasite extracts was adjusted to 1.72 mg/mL.

Immunization and Challenge Procedure

Five groups of 20 rainbow trout were immunized as follows. Fish in Groups 1, 3, 4, and 5 were variously injected (see below) under anaesthesia (tertiary-amyl alcohol) intramuscularly at the base of the dorsal fin using a sterile 1-mL tuberculin syringe fitted with a 25-gauge, 5/8 needle. After injection, fish were allowed to recover in an aquarium tank and were observed for any signs of stress or mortality. No fish died during the immunization procedure. After recovery from the anaesthetic, fish were transferred to their respective tanks. Fish in Group 2 were infected via a stomach tube with two live sealworms according to the procedure described in Ramakrishna and Burt (1991). The five groups were as follows. Group 1: control group injected with 0.5 mL of sterile PBS (pH 7.2), which served as a negative control; Group 2: infected with live larval sealworms; Group 3: injected with 0.5 mL (0.86 mg of protein) of sealworm extract; Group 4: injected with 0.5 mL (0.86 mg of protein) of cestode extract, which served as a positive control; and Group 5: injected with 0.5 mL (0.86 mg of protein) of porcupine pinworm extract, which also served as a positive control.

Two weeks postimmunization (14 DPI), 10 fish in each group were necropsied and the muscle tissue around the site of injection was excised and fixed in Bouin's fluid (15 blocks) for light microscopical studies. The remaining 10 fish in each group were challenged at 14 DPI with live sealworm larvae. Under anaesthesia, each fish was given two worms via a stomach tube as in Ramakrishna and Burt (1991). Two days after this challenge infection, the remaining fish in all five groups were necropsied and the muscle tissue around each worm was excised. A total of 20 worms were fixed and processed for use in light microscopy (15 worms) and electron microscopy (five worms). For electron microscopy, each muscle block was further cut into small cubes of 2 mm^2 and processed. A minimum of three blocks were sectioned and examined for each group. The procedures used for processing tissues for light and electron microscopy are described in Ramakrishna and Burt (1991).

Results

In the control group of fish (Group 1), which received PBS alone, the host tissue response at the site of injection at 14 d posttreatment (DPT) consisted of a mixed population of leucocytes and fibroblasts. Necrosis was very much evident, with loss of muscle structure. The cellular response to a challenge dose (live worms) 2 d postchallenge was similar to the response seen at 2 DPI in previously uninfected or naive fish (see Ramakrishna and Burt 1991), with muscle necrosis, haemor-

rhage, and a predominance of polymorphonuclear neutrophils (PMN) (Fig. 1).

In the second experimental group of fish (Group 2), in which the fish were sensitized with live larvae, the host responses to sealworm at 14 and 16 DPI were similar to the response at 16 DPI in previously uninfected or naive fish. The response in these sensitized fish, at 2 d postchallenge with live sealworm, was similar to the response of naive fish at 2 DPI with respect to intensity and cellular composition. Figure 2 gives a comparative picture of a 16-d-old worm (primary infection) and 2-d-old worm (secondary challenge infection) beside each other in the same fish. The host reactions around each worm were similar.

In the third experimental group of fish (Group 3), in which the fish were immunized with sealworm extract, a nonspecific inflammatory response was seen at 14 DPT at the site of injection. When challenged with live worms 2 wk posttreatment, a stronger immune response was evident at 2 d postchallenge compared with the response evident in Group 2 above. The cellular immune response around worms from Group 3 (Fig. 3) consisted mainly of macrophages and plasma cells compared with a response showing PMNs in Group 2 fish above. Electron microscopical observations confirmed the identity of activated macrophages (Fig. 4 and 5) and plasma cells (Fig. 6). The macrophages were identified as activated macrophages by the presence of active membranes, pseudopodia, and vacuoles. Plasma cells were large and seemed to be actively synthesizing antibodies as indicated by their eccentric nucleus with its prominent nucleolus and cytoplasm packed with rough endoplasmic reticulum.

In the fourth and fifth positive control groups of fish (Groups 4 and 5), which were immunized with nonhomologous extracts of a cestode and another nematode, respectively, a poor secondary immune response to challenge with live sealworm was seen. The 14-d-old lesion at the site of injection in such immunized fish using cestode extract had a severe infiltration of inflammatory cells and granulation tissue, but the response to a challenge dose of live sealworm at 2 d postchallenge was comparable with 2 DPI of naive fish, with a mild infiltration of PMNs and few haemorrhages around the encapsulating worm (Fig. 7). The nonhomologous extract of another nematode showed a similar tissue response at the site of injection and a response to a challenge with live sealworm which was similar to that of a naive fish (Fig. 8).

Discussion

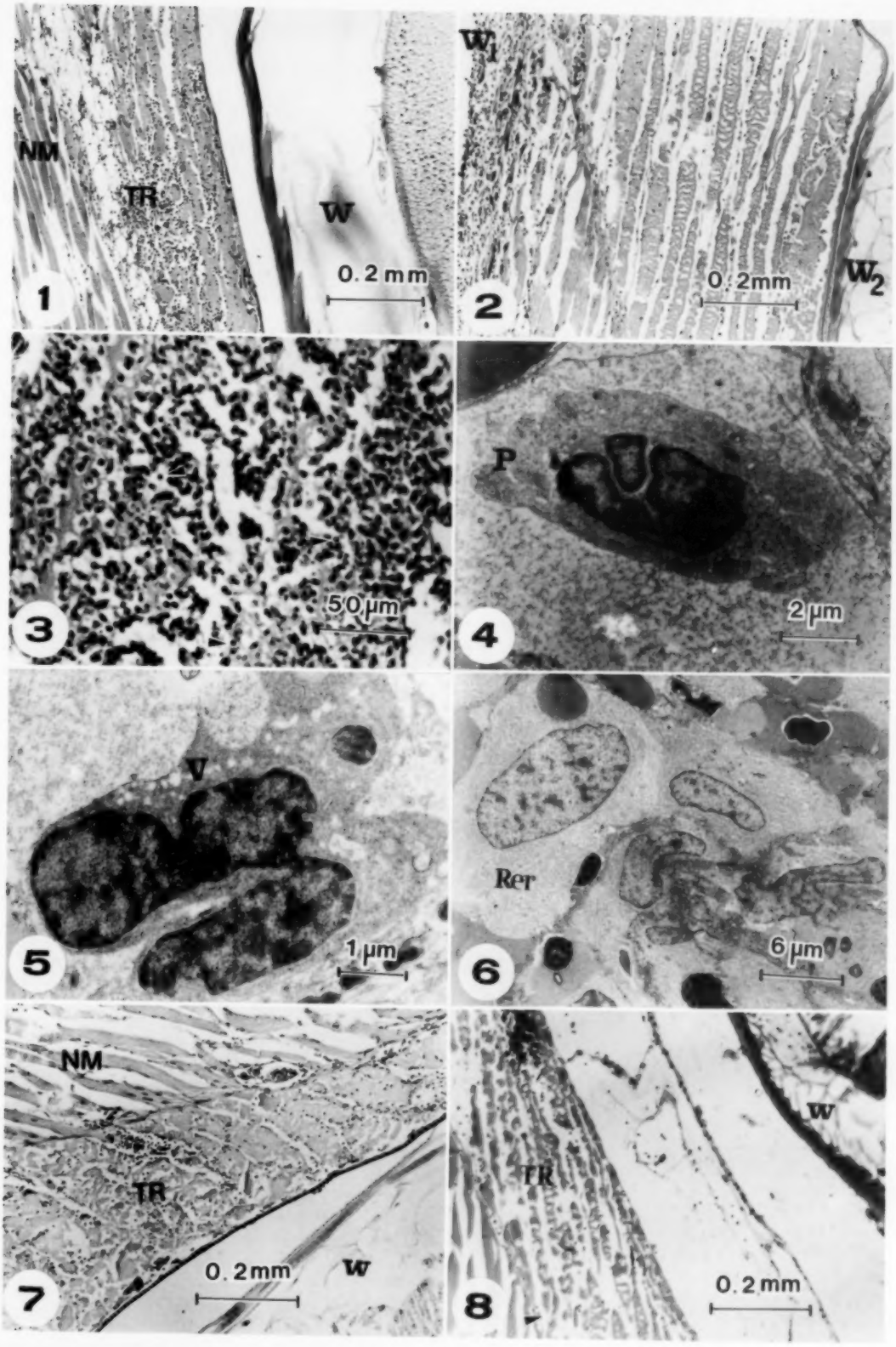
The secondary immune response observed in fish immunized with sealworm extract and challenged 14 DPI with live sealworms was similar to a DTH reaction. Histologically, the classical DTH reaction, in mammals, consists mainly of an infiltration, predominantly of mononuclear cells, into the deep and superficial areas of the dermis. In fish, DTH reactions have also been observed in subdermal tissues, i.e. in muscle (Thomas and Woo 1990a). In the present study the rainbow trout sensitized to sealworm extract produced a typical DTH reaction. These fish produced a significantly stronger secondary response (i.e. postimmunization) to live sealworm than was seen in live worm challenges after either negative (PBS injection) or positive (heterologous cestode and nematode antigen injection) control immunizations. The lesion was mainly infiltrated by macrophages, which were found to be predominantly activated macrophages, and plasma cells. The morphological characteristics of plasma cells indicate the active state of these cells, suggesting antibody production.

The presence of activated macrophages suggests the possible release of lymphokines which is most often associated with T-cell function. The macrophage migration inhibition, which is dependent on migration inhibition factor (MIF, a lymphokine) response, has been demonstrated in vitro in teleosts (McKinney et al. 1976; Ellis 1986; Thomas and Woo 1990a). In vivo studies using bacterial antigen and a mitogen to activate trout peritoneal macrophages have demonstrated the presence of soluble macrophage activating factors (Chung and Secombes 1987; Graham and Secombes 1988). Recently, Whyte et al. (1990) demonstrated the capacity of a diplostomule antigen to activate macrophages in vivo following immunization of rainbow trout with sonicated diplostomules, as indicated by enhanced respiratory burst activity.

When the test fish were immunized with live sealworms and later challenged with live sealworms, the secondary response was poor and did not elicit any heightened response. The response showed no differences between controls and naive fish. This was not surprising. In larger, naturally infected Atlantic cod, it is quite normal to see multistage infections with high intensity. The host response to "early" infections was mild when compared with "old" infections in the same fish as reported earlier (Ramakrishna and Burt 1991). This host response to secondary infections (live worms) in both naturally and experimentally infected fish suggests that the parasite releases antigens slowly, thus delaying a response, or that they are "hidden" from specific immune detection.

In fish, reports of CMI against disease agents are few. A cell-mediated immune response has been demonstrated in rainbow trout to (1) *Mycobacterium tuberculosis* and *M. salmoniphilum* using histological methods which showed a DTH reaction, thymus imprint analysis, and a "skin-window" assay (Bartos and Sommer 1981), (2) *Yersinia ruckeri* by macrophage migration inhibition assay (Blazer and Wolke 1984a, 1984b), and (3) *Cryptobia salmositica* (Thomas and Woo 1990a) using a macrophage migration inhibition assay and DTH reactions. In addition, Graves et al. (1985) reported the pres-

FIGS. 1–8. *Pseudoterranova decipiens* in experimentally infected rainbow trout. Fig. 1. Cellular response at 2 d postchallenge in the control group of fish, which received PBS. The response was dominated by PMNs. NM = normal muscle, TR = tissue response, W = worm. Fig. 2. Micrograph showing the cellular response in the experimental group of fish in which fish were sensitized with live larvae, the host response around a 16 DPI worm (W_1, primary infection), and a 2 d postchallenge worm (W_2, secondary infection). Notice the absence of heightened secondary response. Fig. 3. Cellular immune response following an injection of crude extract of sealworm. The section shows the heightened secondary response around the worm at 2 d postchallenge, with the increased infiltration of macrophages and plasma cells (plasma cells indicated with arrowheads). Fig. 4 and 5. Electron micrographs of activated macrophages around the worm at 2 d postchallenge following an injection of crude extract of sealworm. Notice the pseudopodia and vacuoles which are the characteristics of an activated macrophage. P = pseudopodium, V = vacuoles. Fig. 6. Electron micrograph of plasma cells at the site of infection at 2 d postchallenge following injection of crude extract. The plasma cells were active, each with a prominent nucleolus and dilated cisternae of endoplasmic reticulum. Rer = rough endoplasmic reticulum. Fig. 7 and 8. Micrographs showing the poor secondary response at 2 d postchallenge in the control groups of fish which were immunized with nonhomologous extracts of a cestode (Fig. 7) and another nematode (Fig. 8). NM = normal muscle, TR = tissue response, W = worm.


NM
TR
W
0.2 mm
1
W1
0.2mm
W2
2
5.0 µm
3
P
2 µm
4
V
1 µm
5
Rer
6 µm
6
NM
TR
0.2mm
W
7
W
TR
0.2 mm
8

ence of nonspecific cytotoxic cells in channel catfish (*Ictalurus punctatus*) to *Tetrahymena pyriformis*.

In fish immunized against bacteria and helminths, researchers have failed to correlate protection with specific antibody titre alone (Michel and Faivre 1982; McCarthy et al. 1983; Whyte et al. 1990). It was suggested that a circulating antibody was not the only mechanism involved in protection and that a synergistic interaction between activated macrophages and specific antibodies was implicated in conferring immunity.

In mammals, activated macrophages have been implicated as effector cells in defence against multicellular parasites such as *Schistosoma mansoni* (Cohen and Warren 1982; Capron et al. 1983). Activation of macrophages involves significant changes in cell surface receptors and cellular metabolism, including enhanced bactericidal activity associated with increased production of reactive oxygen species (ROS) such as O_2^-, H_2O_2, and OH. Similarly, in trout, activation of macrophages for increased bactericidal activity has been demonstrated in vivo (Olivier et al. 1986; Graham et al. 1988) and in vitro (Graham and Secombes 1988). However, activation of trout macrophages alone does not increase larvicidal activity for diplostomules in vitro unless the larvae were opsonized with immune serum (Whyte et al. 1989). Larvicidal activity against diplostomules has been demonstrated in vivo (Whyte et al. 1990), suggesting a synergistic interaction between activated macrophages and specific antibodies. Thus, antibody-dependent cell-mediated cytotoxicity (ADCC) has been suggested as the possible effector mechanism in conferring protection against fish helminths (Whyte et al. 1990). The results of the present study, showing the presence of activated macrophages and plasma cells, also suggest the possibility of interaction between macrophages and specific antibodies. Clearly, more work is needed to elucidate the relationship between activated macrophages and specific antibodies in sealworm infections.

In conclusion, the present experiment suggested that *P. decipiens* was capable of eliciting a cell-mediated immune response in fish immunized with somatic antigens (whole-worm extract) as manifested by the DTH reaction. In contrast, when immunized with live intact worms the secondary response did not exhibit any anamnesis under the conditions tested. The long survival of *P. decipiens* within an immunologically competent and sensitized natural host, such as Atlantic cod, reflects its ability to avoid potentially lethal consequences of immune sensitization. In mammals, a variety of immune evasion mechanisms, such as anatomical seclusion of parasites, variation, shedding or disguise, or slow release of surface antigens and the modification of host immune responsiveness, have been recognized and described. Further research is needed to elucidate the survival mechanism of sealworm in a competent host.

The ability of sealworm extract to elicit an immune response suggests several areas which should be explored: (1) the identity of the antigen(s) responsible for the immune response, (2) whether such antigen(s), are released by intact worms in the flesh of the host, (3) whether the intact worm also produces immunosuppressive factors, or (4) whether the response elicited is a function of the amount and availability of antigen involved (i.e. relatively little from intact worms; large amounts in injected homogenized extracts).

Acknowledgements

The authors gratefully acknowledge the help of Ms. C. G. Likely and Mr. C. Keeling with the experimental infections. Financial assistance was provided by the Natural Sciences and Engineering Research Council of Canada through their University–Industry Program (to M. D. B. Burt), the University of New Brunswick, Atlantic Salmon Disease Consortium, and the Department of Fisheries and Oceans. The senior author was supported during part of this study by a Commonwealth Scholarship and Fellowship Plan. The work was done at the Huntsman Marine Science Centre with the able assistance of Mr. F. Purton.

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Prey Aggregation Is Correlated with Increased Predation Pressure in Lake Fish Communities

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Johannes, M. R. S. 1993. Prey aggregation is correlated with increased predation pressure in lake fish communities. Can. J. Fish. Aquat. Sci. 50: 66–73.

Aggregations of prey fish, golden shiner (*Notemigonus crysoleucas*), were examined during 7 yr of predator manipulations in two lakes to determine whether they responded to changes in predation pressure and varied with time-of-day, age, and habitat. Regression analysis was used to examine aggregation in 12 replicate prey densities from two time periods, two ages, two habitats, three sample series, and seven predator densities. Aggregation was assessed as the variance of mean densities for each treatment combination. Multiple regression and ANCOVA analyses indicated that (1) golden shiner aggregated more during day than night, (2) their aggregation was positively related to predator density, (3) young shiner aggregated more than older ones at low predator densities, and (4) aggregation in older shiner was more responsive to increased predator densities than aggregation in younger shiner. These results provide empirical evidence that golden shiner aggregation patterns respond to predation pressure and the response varies with time and age. These results also suggest that variance in net catches can provide an index of fish aggregation and that aggregation observed at the population level is not solely dependent on species and density, but is a behavioural response mediated by several factors including predators.

Au cours des sept années d'expériences effectuées sur des prédateurs vivant dans deux lacs, nous avons étudié les concentrations d'un poisson proie, la chatte de l'est (*Notemigonus crysoleucas*), afin de déterminer si cette espèce réagissait à la pression exercée par la prédation et si les concentrations variaient en fonction du moment de la journée, de l'âge et de l'habitat. À l'aide d'analyses de régression, nous avons étudié sur des réplicats les concentrations pour douze densités de proies échantillonnées à deux moments différents, parmi deux groupes d'âge, en provenance de deux habitats, au cours de trois séries d'échantillonnage et en présence de sept densités de prédateurs. Nous avons évalué la concentration parallèlement à la variance de la densité moyenne pour chaque combinaison étudiée. Les analyses de régression multiple et les analyses de covariance indiquent que 1) les concentrations de chatte de l'est sont plus fortes le jour que la nuit, 2) la concentration est directement proportionnelle à la densité des prédateurs, 3) en présence d'une faible densité de prédateurs, les concentrations de juvéniles sont supérieures à celles des individus plus âgés et 4) les concentrations des individus plus âgés sont davantage influencées par l'augmentation de la densité de prédateurs par rapport à celles des jeunes chattes de l'est. Ces résultats constituent des preuves empiriques que la pression exercée par les prédateurs influe sur les concentrations de la chatte de l'est et que la réponse de l'espèce à cette pression varie en fonction du moment de la journée et de l'âge. Ces données laissent également penser que la variance dans les captures au filet peut fournir un indice de la concentration des poissons et que la concentration observée dans la population ne dépend pas seulement de l'espèce et de la densité mais constitue une réponse volontaire découlant de plusieurs facteurs, notamment les prédateurs.

Received September 23, 1991
Accepted July 9, 1992
(JB227)

Reçu le 23 septembre 1991
Accepté le 9 juillet 1992

Although there has been a long-held belief that prey aggregate in response to predators, empirical tests of this hypothesis in nature are almost entirely lacking in the literature (but see Rasmussen and Downing 1988; Lima and Dill 1990). In the presence of predators, prey form aggregations as behavioural refuges against predator attack (Neill and Cullen 1974; Poole and Dunstone 1975; Milinski 1979; Tremblay and Fitzgerald 1979; Magurran and Pitcher 1987). However, in the absence of predators, larger aggregations of prey lead to increased resource competition (Willis 1972; Eggers 1976; Bertram 1978; Major 1978; Pulliam and Caraco 1984; Street et al. 1984; Pitcher 1986).

Studies of fish behaviour have indicated that aggregated prey have greater individual survival than solitary prey (Radakov 1973; Neill and Cullen 1974; Major 1978; Morgan and Godin 1985; Landeau and Terborgh 1986; Pitcher 1986). The factors involved include (1) minimization of individual detection by and contact with predators (Helfman and Schultz 1984; Helfman 1986; Pitcher 1986), (2) reduced probability of individual attack (Neill and Cullen 1974; Keenleyside 1979; Morgan and Godin 1985), (3) experience gained in evading attack at little individual cost (Turner and Pitcher 1986), (4) coordination of escape tactics (Andorfer 1980; Godin and Morgan 1985), (5) confusion of predators through the presentation of multiple potential targets (Neill and Cullen 1974; Major 1978; Milinski 1979), and (6) increased probability of early detection of predators (Magurran et al 1985; Godin et al. 1987).

Aggregated individuals also may gain benefits while foraging by (1) faster location of food (Pitcher et al. 1982),

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(2) increased duration of feeding time (Magurran and Pitcher 1983), (3) more effective sampling of the environment (Pitcher and Magurran 1983), and (4) improved information transfer among group members (Magurran 1984). While these benefits may be realized by small-sized aggregations, with increased group sizes, competition quickly intensifies to outweigh aggregation-related foraging benefits (Caraco and Wolf 1975; Eggers 1976; Bertram 1978; Major 1978; Nudds 1978; Barnard 1980; Pulliam and Caraco 1984; Street et al. 1984; Pitcher 1986).

A widely held hypothesis states that aggregation in prey appears to have evolved as a response serving to limit predation; however, this is supported by little quantitative field evidence at the population level (Taylor et al. 1978; Hanski 1982; Gilinsky 1984; Taylor 1984; Pinel-Alloul et al. 1988; Rasmussen and Downing 1988; Lima and Dill 1990). In this study, I investigate how patterns of aggregation in populations of prey fish, golden shiner (*Notemigonus crysoleucas*), are correlated with changes in predation pressure. Predator abundances were manipulated in two lakes, providing an opportunity to examine the relationship between prey aggregation and predators in nature.

This study focuses on golden shiner because field observations demonstrate that their behaviour and survival are strongly influenced by predators, depending on age class (Johannes et al. 1989; Johannes 1990). An enclosure study showed that young shiner avoided increasing levels of predation pressure through increased aggregation and use of artificial shelter, while older prey only aggregated (Johannes 1990). In enclosure and field studies, shiner have been observed schooling during the day and dispersing and feeding at dusk (Hall et al. 1979; Johannes 1990). Combined, these observations suggest that patterns of aggregation in shiner can be related to the effects of predators among different age classes, times-of-day, and habitats. Alternately, aggregation may simply be a species-specific function related to density, as suggested for many other animals species (Taylor 1984). Therefore, the purpose of this study was twofold: (1) to test the hypotheses that aggregation in golden shiner responds to changes in predation pressure and varies with time-of-day, age, and habitat and (2) to determine whether the level of golden shiner aggregation among times, ages, and habitats can be predicted with changes in predator density.

Methods

Tests of these hypotheses were performed by analyzing variation among samples of two ages of golden shiner collected at day and night, in inshore and offshore habitats, and during three different sample series over the course of predator manipulations in two lakes: Lake St. George, Ontario, and Wintergreen Lake, Michigan. Fish were restricted to the top 4 m of the water column of both lakes by anoxic (hypolimnetic) water below these depths. Fish communities in both lakes consisted of prey fishes including golden shiner, yellow perch (*Perca flavescens*) and pumpkinseed (*Lepomis gibbosus*) and predator fishes including largemouth bass (*Micropterus salmoides*), northern pike (*Esox lucius*), and walleye (*Stizostedion vitreum*) in Lake St. George and largemouth bass, bowfin (*Amia calva*) and grass pickerel (*Esox americanus vermiculatus*) in Wintergreen Lake. Abundances of individual predator species were estimated using multiple census mark–recaptures conducted annually during August and September. Details of these methods are given in Johannes et al. (1989) and McQueen et al. (1989) for Lake St. George and in Johannes (1990) for Wintergreen Lake.

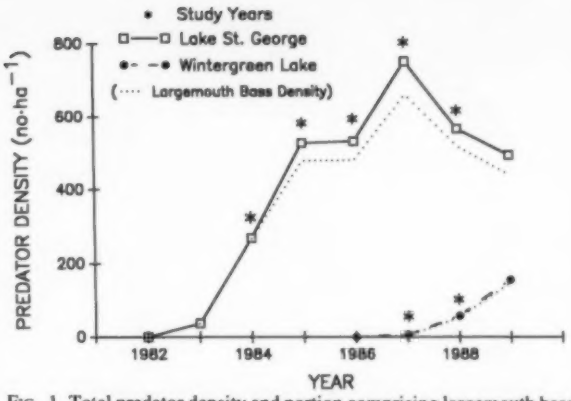

FIG. 1. Total predator density and portion comprising largemouth bass density estimated from August and September census periods during 1982–89 in Lake St. George and Wintergreen Lake. Asterisks indicate study years used for analysis of prey aggregation. Data from Johannes et al. (1989) and McQueen et al. (1990) for Lake St. George and from Johannes (1990) for Wintergreen Lake.

During the course of the study, predator abundances increased threefold in Lake St. George from 1984 to 1988 and 10-fold in Wintergreen Lake from 1987 to 1988 (Fig. 1). There was a difference of greater than 100-fold between lakes (6–750 predators·ha^{-1}). In both lakes, predator communities were dominated by largemouth bass. Predator manipulations were initiated as a result of natural predator winterkills in Lake St. George during 1982 (Johannes et al. 1989; McQueen et al. 1989) and in Wintergreen Lake during 1977 and 1978 (Hall and Ehlinger 1989; Johannes 1990). The winterkill in 1982 in Lake St. George killed more than 90% of the predators. By 1984, predator densities began to increase through a combination of stocking and natural recruitment. All predators were lost in Wintergreen Lake through winterkills and were absent from the lake until reintroduced through stocking in 1987 (Hall and Ehlinger 1989; Johannes 1990).

Sampling was conducted throughout August and September during 1984–88 in Lake St. George and 1987–88 in Wintergreen Lake. Inshore and offshore habitats were sampled using a beach seine (31.5 × 2 m, 1-mm^2 mesh) and a purse seine (31.5 × 9.8 m, 1-mm^2 mesh). Nets were constructed to standardize the surface area of water fished to approximately 100 m^2. Each sample series consisted of 12 replicate samples of golden shiner collected during the day (10:00–16:00) and 12 during the night (23:00–04:00). Sample series were completed as quickly as physically possible, always within 48 h, and repeated three times, separated by at least 10 d, for each time, habitat, and predator density combination. If greater than 12 samples were collected in a series, then 12 replicates were randomly selected out of the larger set of observations to produce equal numbers of replicates among all sample series. Young-of-year (YOY) and older than YOY (>YOY) golden shiner were counted within each sample. Sample sites within each habitat were randomly selected from a set of 40 possible inshore and offshore sites in each lake during each sample series. These methods resulted in 168 overall estimates of golden shiner density derived from both lakes, 12 replicate samples within two age groups, two time periods, two habitats, three sample series, and seven predator densities (i.e. total samples = 12 replicates × 2 ages × 2 times × 2 habitats × 3 series × 7 predator densities). From a total of 2016 net samples, 168 observations of mean density were derived, and only 129 with values of

TABLE 1. Multiple regression analysis of golden shiner aggregation ($\log_{10}$ variance = dependent variable) using a model that incorporates mean density ($\log_{10}$ mean) and coded independent class variables for time-of-day (day = 1, night = 0), age (>YOY = 1, YOY = 0), habitat (inshore = 1, offshore = 0), sample series (two variables, A and B: first sample series = 1,0; second = 0,1; third = 0,0), and predator density (PD (no.·ha^{-1}) from Fig. 1). Initial multiple regression model: $n = 129$, $F = 480.8$, $r^2 = 0.97$, $P < 0.0001$. Final multiple regression equation using variables from initial multiple regression (stepwise analysis) that predicted a significant portion of the variation in aggregation: $n = 129$, $F = 841.9$, $r^2 = 0.96$, $P < 0.0001$). Coefficients (SE = standard error) are the fitted regression coefficients for each independent variable; the t value, probability (P) level, and partial correlation coefficient (r) indicate the significance and contribution of each variable to the model for the prediction of $\log_{10}$ variance. NS = variable not significant at $P \leq 0.05$.

Variable	Coefficient	t value	P	Partial r
	Initial multiple regression model			
Mean	1.80	49.6	<0.0001	0.95
Time-of-day	0.94	18.8	<0.0001	0.74
Age	0.12	2.4	0.024	0.05
Habitat	−0.07	−1.2	0.23	0.01 NS
Sample series A	−0.08	−1.1	0.26	0.01 NS
Sample series B	−0.04	−0.5	0.62	0.001 NS
Predator density	0.001	9.7	<0.0001	0.43
	Final multiple regression equation			
Mean	1.78 (0.03)	52.9	<0.0001	0.96
Time-of-day	0.94 (0.05)	19.0	<0.0001	0.75
Age	0.12 (0.05)	2.4	0.018	0.044
Predator density	0.13×10^{-3} (1.1×10^{-4})	9.7	<0.0001	0.43
Intercept	−0.40 (0.08)			

$M > 0$ were considered in this analysis. Mean golden shiner densities (M) had a range of 0.0–425.3 per net, and variances (S^2) had a range of 0.0–192 674 within ages, times, habitats, and predator densities.

The 12 replicate golden shiner density estimates were taken at randomly chosen points in space during each sample series; therefore, the variation among estimates is expected to be higher if aggregation is higher in the population (e.g. Downing 1979, 1986; Pinel-Alloul et al. 1988; Rasmussen and Downing 1988; Pace et al. 1991). I tested my first set of hypotheses that golden shiner aggregation responds to changes in predation pressure and varies with time-of-day, age, sample series, and habitat by deriving estimates of mean (M) and variance (S^2) within each time – age – habitat – sample series – predator density combination and then using the variance as the dependent variable in multiple regression analysis (cf. Pinel-Alloul et al. 1988). The form of the regression model used was

$$\log_{10}S^2 = a + b\log_{10}M + c\text{TOD} + d\text{AGE} + e\text{HAB} + f\text{SS-A} + g\text{SS-B} + h\text{PD} + (\text{error})$$

where TOD is the coded time-of-day (day = 1, night = 0), AGE is the coded age (>YOY = 1, YOY = 0), HAB is the coded habitat (inshore = 1, offshore = 0), SS-A and SS-B are the coded two-variable sample series (first = 1,0; second = 0,1; third = 0,0), PD is predator density (6, 59, 269, 527, 532, 566, 751·ha^{-1}), and a to h are fitted coefficients of the regression (e.g. Pinel-Alloul et al. 1988; Pace et al. 1991). Logarithmic transformations were used to reduce heteroscedasticity of variance of S^2 at any given values of M. Inclusion of independent variables in the model predicting S^2 was based on a stepwise test of the significance of the t value associated with each regression coefficient. Interactions among variables in the multiple regression model were evaluated using partial residual analysis and the significance of partial correlation coefficients. This analysis also determined the sole contribution of each independent variable toward prediction of the dependent variable. The contribution of between-lake and between-year differences to the prediction of aggregation was checked using the residuals generated from the final multiple regression model compared by simple analysis of variance (ANOVA) among lakes, years, and lake–year interactions.

I tested my second hypothesis, that the level of aggregation among times, ages, and habitats can be predicted with changes in predator density, by fitting the function $S^2 = aM^b$ (M and S^2 are the same as above and a and b are fitted coefficients for this relationship (Taylor 1984; Downing 1986; Rasmussen and Downing 1988)) within each time, age, habitat, sample series, and predator density and testing for differences among regression coefficients. Times, ages, habitats, sample series, and predator densities were used in the test of the second hypothesis based on their inclusion in the final regression model in the test of the first hypothesis. An analysis of covariance (ANCOVA) of $\log_{10}S^2$, with $\log_{10}M$ as a covariate, was used to test for coincidental $\log_{10}S^2$–$\log_{10}M$ relationships for each time, age, habitat, sample series, and predator density and to test for interactions among these categories of data. I used multiple regression analyses to predict the relationship between the level of aggregation and predator density based on differences between $\log_{10}S^2 = a + b\log_{10}M$ relationships among time, age, sample series, and habitat. I assumed that the $\log_{10}S^2$–$\log_{10}M$ relation-

TABLE 2. Two-way ANOVA of residuals (predicted minus actual $\log_{10}$ variance), from the final regression model in Table 1, compared among lakes ($n = 2$) and years ($n = 5$). Total $n = 129$.

Factor	df	SS	F	P
Model	6	0.57	1.37	0.24
Lake	1	0.0027	0.039	0.84
Year	4	0.56	2.02	0.25
Lake × year	1	0.0039	0.056	0.81
Error	122	8.44		

ships with the highest S^2 at any given M were the most aggregated. These analyses make no assumptions about the relationship between b and level of aggregation (Downing 1986, 1989; Rasmussen and Downing 1988). All analyses were performed using SAS (Freund and Littell 1986). Statistical significance was concluded at $P \leq 0.05$.

Results and Discussion

Golden shiner were more aggregated during the day than at night, and the level of aggregation during the day is correlated with predation pressure (Tables 1, 2, 3, 4). Younger shiner (YOY) were more aggregated than the older fish (>YOY) at low predator densities and did not respond as much as older shiner to increased predation pressure (Table 5). Patterns of golden shiner aggregation in Lake St. George and Wintergreen Lake are similar to those in enclosures (Johannes 1990), in other aquatic systems (Zaret and Suffern 1976; Hall et al. 1979; Helfman 1981), and for other animal species (e.g. Hobson 1965; Major 1978; Caraco et al. 1980; Gilinsky 1984; Rasmussen and Downing 1988; Turchin and Kerciva 1989). These observations support the hypothesis that golden shiner aggregation behaviour is responsive to predation pressure; they are consistent with results of other studies indicating that behaviour of prey species is flexible in response to the magnitude of perceived risk from predators (Barnard 1980; Curio et al. 1983; Dill and Fraser 1984; Giles and Huntingford 1984; Lima 1985; Milinski 1986; Gilliam and Fraser 1987; Abrahams and Dill 1989; McLean and Godin 1989).

Multiple regression analysis indicates that time-of-day, age, and predator density, in addition to golden shiner density (M), account for significant variation in the prediction of their aggregation (Table 1). Different lakes and years fit equally to this regression model (Table 2), and their inclusion in this model does not improve the prediction of aggregation. Sample series and habitat are not significant predictors of aggregation, indicating that aggregation did not change significantly during the course of the study and was similar between inshore and offshore habitats. Subsequent analyses were split among day and night class variables because of the large contribution of time-of-day to prediction of aggregation.

ANCOVA indicates that age and predator density had significant effects on the level of golden shiner aggregation (S^2) at given mean density (M, covariate) during the day (Table 3), but not at night (Table 4). These analyses indicate that predictions of aggregation can be separated into three significant relationships: separately for YOY and >YOY during the day and for combined ages at night. These results represent three separate regression models: (1) YOY prey during the day (Table 5), (2) >YOY prey during the day (Table 5), and (3) both ages of prey at night (Table 6). Multiple regression models for YOY and >YOY fish during the day (Table 5) indicate that shiner density (M) and predator density (PD) account for significant

TABLE 3. Summary of ANCOVA analysis of a model of golden shiner aggregation ($\log_{10}$ variance = dependent variable) during the day using a model that incorporates mean density ($\log_{10}$ mean, covariate) and the class variables age (>YOY = 1, YOY = 0) and predator density (PD (no.·ha^{-1}) from Fig. 1) and including 2 × 2 interactions among class variables. Model: $n = 63$, $F = 154.4$, $P < 0.0001$). For each variable, SS = sum of squares, $F = F$ value, and P = probability level. S indicates that significance was found at $P \leq 0.05$.

Variable	SS	F	P	Significance
Mean	129.1	2978.2	<0.0001	S
Predator density	9.9	38.1	<0.0001	S
Age	0.46	10.6	0.0022	S
Mean × PD	0.64	2.45	0.041	S
Mean × age	0.02	0.50	0.49	
PD × age	0.63	2.42	0.043	S
Error	1.78			

TABLE 4. Summary of ANCOVA analysis of a model of golden shiner aggregation ($\log_{10}$ variance = dependent variable) during the night using a model that incorporates mean density ($\log_{10}$ mean, covariate) and the class variables age (>YOY = 1, YOY = 0) and predator density (PD (no.·ha^{-1}) from Fig. 1) and including 2 × 2 interactions among class variables. Model: $n = 66$, $F = 173.9$, $P < 0.0001$). For each variable, SS = sum of squares, $F = F$ value, and P = probability level. S indicates that significance was found at $P \leq 0.05$.

Variable	SS	F	P	Significance
Mean	96.9	3595.2	<0.0001	S
Predator density	0.27	1.68	0.15	
Age	0.039	1.45	0.24	
Mean × PD	0.32	1.99	0.094	
Mean × age	0.09	3.36	0.080	
PD × age	0.33	2.07	0.081	
Error	1.18			

variation in the prediction of prey aggregation. YOY showed less of an aggregation response to predators (slope) but had a greater intercept and therefore showed a greater level of aggregation at low predator density. At night, however, only shiner density (M) accounts for significant variation in the prediction of aggregation, while age and predator density are not significant predictors (Table 6).

Prey reduce levels of aggregation between day and night because lower light levels at night limit the visual ability of prey to coordinate in groups (Shaw 1961; John 1964; Tibbo 1965; Whitney 1969; Blaxter 1970; Radakov 1973; Hunter and Nicholl 1985; Glass et al. 1986) and reduce risk from predators by limiting the visual ability of predators (Dawkins and Krebs 1979; Helfman 1986; Johannes 1990). Golden shiner are not well adapted for night vision (Allan and McFarland 1973); therefore, the overall level of aggregation at night is probably restricted by limits in vision. The risk of predation on golden shiner is minimal at night (Johannes 1990), which may allow night aggregation to be more sensitive to other factors including the availability and spatial distribution of food.

Twilight feeding activities in golden shiner coincide with peak abundances in vertically migrating zooplankton prey (Hall et al. 1979; M. R. S. Johannes, D. J. Hall, and D. J. McQueen, unpubl. data). Levels of aggregation at night may be responsive to the spatial distribution of planktonic food resources because foraging activities can benefit at lower levels of aggregation from cooperation among individuals (for review, see Magurran 1990).

TABLE 5. Multiple regression analysis of golden shiner aggregation ($\log_{10}$ variance = dependent variable) during the day using a model that incorporates mean density ($\log_{10}$ mean) and predator density (PD (no.·ha^{-1}) from Fig. 1) for prey older than YOY (>YOY) ($n = 36$, $F = 608.4$, $r^2 = 0.97$, $P < 0.0001$) and YOY prey ($n = 27$, $F = 889.6$, $r^2 = 0.99$, $P < 0.0001$).

Variable	Coefficient (SE)	*t* value	*P*	Partial *r*
>YOY				
Mean	1.78 (0.06)	32.1	<0.0001	0.97
Predator density	0.0021 (0.00017)	12.3	<0.0001	0.82
Intercept	0.0			
YOY				
Mean	1.72 (0.04)	42.2	<0.0001	0.98
Predator density	0.00086 (0.0001)	6.65	<0.0001	0.65
Intercept	0.60 (0.08)			

TABLE 6. Multiple regression analysis of golden shiner aggregation ($\log_{10}$ variance = dependent variable) during the night combined for both YOY and >YOY using a model that incorporates mean density ($\log_{10}$ mean) and predator density (PD (no.·ha^{-1}) from Fig. 1) for the initial multiple regression model ($n = 66$, $F = 561.0$, $r^2 = 0.96$, $P < 0.0001$) and the final regression equation using variables from the initial multiple regression (stepwise analysis) that predicted a significant portion of the variation in aggregation ($n = 66$, $F = 1681.4$, $r^2 = 0.96$, $P < 0.0001$). NS = variable not significant at $P \leq 0.05$.

Variable	Coefficient (SE)	*t* value	*P*	Partial *r*
Initial multiple regression model				
Mean	1.60	29.4	<0.0001	0.93
Age	0.05	0.86	0.39	0.012 NS
Predator density	0.00019	1.11	0.27	0.02 NS
Final regression model				
Mean	1.56 (0.05)	41.0	<0.0001	
Intercept	0.21 (0.05)			

Average levels of aggregation for zooplankton are similar to average levels of aggregation in golden shiner at night but are much lower than levels in golden shiner during the day (Fig. 2). A regression analysis suggests that aggregation at night is negatively related to the availability of zooplankton prey (Table 7). These results imply that daytime aggregation is primarily used as a predator avoidance behaviour, but at low nighttime levels of predation pressure, aggregation behaviour may be sensitive to several other factors, including food availability.

Differences in aggregation responses to predators between YOY and >YOY golden shiner may be related to size and vulnerability to predation. Smaller-sized, younger fish had higher mortality rates (Johannes et al. 1989; Johannes 1990) and were more aggregated at lower predator densities. This suggests that smaller prey may perceive greater predation risk relative to larger, older prey. Because larger prey are less vulnerable to predators, they may be able to reduce levels of aggregation in order to limit competition for food related to aggregation (cf. Werner and Gilliam 1984). The increase in aggregation of smaller golden shiner may be less pronounced in response to increased predation pressure, relative to larger prey, because the former often select sheltered habitats (Johannes 1990; cf. Werner et al. 1983). Smaller prey may not benefit as much as larger prey from aggregation to avoid predators. Swimming speed and therefore size are probably important components of aggregation behaviour and its effectiveness in avoidance and escape from predators (e.g. Radakov 1973; Musinic 1977; Pitcher et al. 1986). My observations of golden shiner behaviour in enclosures and in the field are supported by similar observations of centrarchids which demonstrate that smaller-sized prey often avoid predators by inhabiting shelter, while larger, less vulnverable fish occupy open-water habitats (Mittelbach 1981; Savino and Stein 1982, 1989; Werner et al. 1983).

Studies of the functional significance of aggregation behaviour suggest that increased aggregation confers increased probability of individual survival (Radakov 1973; Charnov and Krebs 1975; Bertram 1978; Kenward 1978; Pulliam and Caraco 1984; Pitcher 1986). Four studies which have quantified survival of small bony-rayed fishes at different levels of aggregation in the presence of predators (Neill and Cullen 1974; Major 1978; Morgan and Godin 1985; Landeau and Terborgh 1986) indicate that the probability of capture of an individual decreases with increased aggregation (Fig. 3). In this study, I

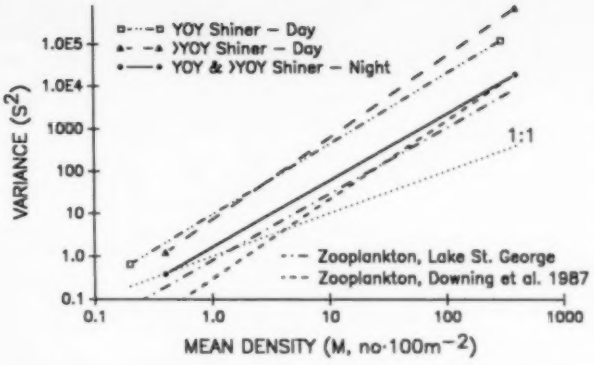

FIG. 2. Contrast between models of aggregation ($\log_{10}$ variance/$\log_{10}$ mean relationships) from (1) YOY golden shiner during the day, (2) >YOY during the day, (3) YOY and >YOY at night (from Table 6), (4) average zooplankton density from Lake St. George, 1982 (Malone 1986), (5) average zooplankton density from freshwater and marine systems (Downing et al. 1987), and (6) mean = variance line (1:1) representing a random distribution. Average zooplankton density regression lines were adjusted to reflect similar spatial scales based on a regression coefficient for scale from a model of zooplankton aggregation derived by Pinel-Alloul et al. (1988).

TABLE 7. Multiple regression analysis of golden shiner aggregation ($\log_{10}$ variance = dependent variable) during the night combined for both YOY and >YOY using a model that incorporates mean density ($\log_{10}$ mean) and availability of zooplankton prey (biomass, mg·L^{-1}). Model: $n = 66$, $F = 969.1$, $r^2 = 0.97$, $P < 0.0001$). Zooplankton data for Lake St. George are from Johannes et al. (1989) and McQueen et al. (1989, 1990) and for Wintergreen Lake from Johannes (1990).

Variable	Coefficient (SE)	*t* value	*P*	Partial *r*
Mean	1.61 (0.05)	42.0	<0.0001	0.97
Zooplankton biomass	−0.064 (0.01)	−3.22	0.002	0.14
Intercept	0.38 (0.07)			

have shown that increased aggregation of golden shiner is correlated with increased predator densities; however, my results cannot determine whether this species attained a greater survival through increased aggregation.

Patterns of golden shiner presence in various natural aquatic systems indicate that this prey fish survives better than many similar morphs of prey (e.g. bluntnose minnow (*Pimephales notatus*), fathead minnow (*Pimephales promelas*), brassy minnow (*Hybognathus hankinsoni*), northern redbelly dace (*Phoxinus eos*), finescale dace (*Phoxinus neogaeus*), and blacknose shiner (*Notropis heterolepis*)) in fish communities containing dense predator populations (Tonn and Magnuson 1982; Rahel 1984; He and Kitchell 1990; Johannes 1990). Aggregation behaviour in golden shiner is responsive to changes in predation pressure, which may lead to improved survival in a variety of physically and biologically different communities (e.g. Rahel 1984; He and Kitchell 1990).

I have demonstrated here that aggregation in two freshwater populations of golden shiner is not only related to population density but is correlated with predation pressure and varies with time-of-day and age of fish. In this study, I have also quantified the level of aggregation as a function of changing predator densities in the day and at night for two age classes of shiners. My results illustrate that aggregation in fish can be indexed through use of estimates of variance in net samples. The agreement of my results, using fish, with results of studies of aggregation in other organisms (e.g. Downing 1979; Pinel-Alloul et al. 1988; Rasmussen and Downing 1988; Pace et al. 1991) suggests that spatial distribution in aquatic animals is not a species-specific trait solely related to density. Rather, aggregation is variable and is determined by environmental variables such as predators and food, interacting with age and the diurnal cycle.

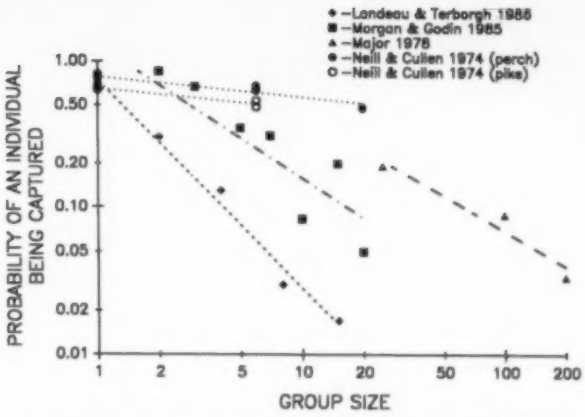

FIG. 3. Literature-derived relationships between the probability of an individual prey fish being captured and group size. The data are derived from (1) Landeau and Terborgh (1986), five points using Mississippi silvery minnow (*Hybognathus nuchalis*) as prey and largemouth bass (*Micropterus salmoides*) as predator (correlation: $r^2 = -0.99$, $P < 0.0001$); (2) Morgan and Godin (1985), eight points, using banded killifish (*Fundulus diaphanus*) as prey and white perch (*Morone americana*) as predator (correlation: $r^2 = -0.79$, $P < 0.0001$); (3) Major (1978), three points, using Hawaiian anchovy (*Stolephorus purpureus*) as prey and jack (*Caranx ignobilis*) as predator (correlation: $r^2 = -0.94$, $P < 0.0001$); and (4) Neill and Cullen (1974), 14 points, using bleak (*Alburnus alburnus*), dace (*Leuciscus leucuiscus*), guppy (*Poecilia vivipara*) and minnow (*Phoxinus phoxinus*) as prey and northern pike (*Esox lucius*) and European perch (*Perca fluviatilis*) as predators (correlation: perch $r^2 = -0.86$, $n = 10$, $P < 0.0001$; pike $r^2 = -0.91$, $n = 4$, $P < 0.0001$). The probability of an individual being captured was measured as the probability of individual capture per attack in studies (1) and (4) and the probability of individual capture over the duration of the experiment in studies (2) and (3).

Acknowledgements

This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to D. J. McQueen and NSERC postgraduate scholarships and postdoctoral fellowships to M.R.S.J. I gratefully acknowledge valuable comments and helpful suggestions provided by John A. Downing, Don J. McQueen, R. Ian Perry, Kim D. Hyatt, Joe B. Rasmussen, and two anonymous referees. I thank Ron Hudson at Lake St. George and Don J. Hall and Joe Johnson at Wintergreen Lake, Michigan State University, for their support throughout this study. I especially thank Dr. John A. Downing whose editorial comments helped improve data analyses and presentation.

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Nutrient and Energy Transport between Estuaries and Coastal Marine Ecosystems by Fish Migration

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Biomass accumulation and changes in body energy and nutrient (carbon, nitrogen, and phosphorus) composition were evaluated relative to the migration pattern of gulf menhaden (*Brevoortia patronus*) to determine if biotic transport by fish is an important source of energy and nutrients to coastal marine ecosystems. Gulf menhaden transported significant quantities of energy, C, N, and P from a Louisiana estuary to the nearshore Gulf of Mexico. Transport was always out of the estuary to the marine system, although the magnitude depended on the balance between growth and mortality rates and abundance of fish. Average export per year was 38 g biomass, 930 kJ, 22.5 g C, 3.1 g N, and 0.9 g $P \cdot m^{-2}$ out of estuaries. This is roughly 5–10% of the total primary production of these estuarine areas. N and P export by fish is of the same magnitude as passive waterborne export; however, it is higher quality. This indicates that fish migration can play an important role in exporting the productivity of estuaries to coastal marine ecosystems.

On a évalué l'accroissement de la biomasse ainsi que les modifications de l'énergie corporelle et de la composition en éléments nutritifs (carbone, azote et phosphore) en fonction du modèle de migration d'une espèce d'alose (*Brevoortia patronus*) afin de déterminer si le transport biotique par les poissons constitue une importante source d'énergie et d'éléments nutritifs dans les écosystèmes marins côtiers. *Brevoortia patronus* transporte des quantités importantes d'énergie, de C, de N et de P d'un estuaire de la Louisiane aux eaux côtières du golfe du Mexique. Ce transport s'effectue toujours de l'estuaire vers l'écosystème marin, bien que son importance dépende de l'équilibre entre les taux de croissance et de mortalité, ainsi que de l'abondance du poisson. La quantité moyenne transportée hors de l'estuaire chaque année correspond à 38 g de biomasse, 930 kJ, 22,5 g de C, 3,1 g de N et 0,9 g de $P \cdot m^{-2}$. Ces chiffres représentent approximativement 5–10 % de la production primaire totale de ces zones estuariennes. L'exportation de N et de F par les poissons a la même importance que l'exportation passive par l'eau, mais elle est de qualité supérieure. Ces résultats indiquent que la migration des poissons peut jouer un rôle important dans l'exportation de la productivité des estuaires vers les écosystèmes marins côtiers.

Received October 25, 1991
Accepted July 9, 1992
(JB281)

Reçu le 25 octobre 1991
Accepté le 9 juillet 1992

Interactions between animals and their environment are now recognized as important influences on the structure and dynamics of ecosystems. The actions of animals, as a result of their food selection, physical disturbance, or movement, can influence a host of processes at the ecosystem level (Kitchell et al. 1979; Carpenter and Kitchell 1988; Naiman 1988; Northcote 1988). Although most studies of ecosystem-level effects have been focused within a single ecosystem, some animals, because of their mobility and their size, can readily cross ecosystem boundaries and effect substantial movement of nutrients (carbon, nitrogen, and phosphorus) and energy.

Anadromous fishes have long been known to provide P to nutrient-poor inland waters (Juday et al. 1932; Krokhin 1975; Durbin et al. 1979; Stockner 1987; Northcote 1988; Kline et al. 1990). Fish migrations also promote heterotrophic activity and periphyton production (Rickey et al. 1975; Durbin et al. 1979) and maintain the P reserves (Hall 1972). Smith (1966) has suggested that the migration of catadromous fishes might represent appreciable organic matter losses from lakes.

Largely as a result of the conception that physical processes dominate biogeochemical cycles, little attention has been paid to the potential of animal-mediated cycling of nutrients in marine ecosystems. The enormous movements of water by tides and freshwater inflows are thought to control the flux of nutrients and energy in estuaries (Day et al. 1989). However, fish also represent an important link between estuaries and offshore ecosystems, although no one has evaluated their role in nutrient movement. Estuarine fish faunas around the world are dominated in numbers and biomass by species which move into the estuary as larvae, accumulate biomass, and then move offshore after attaining a large proportion of their adult size (Yáñez-Arancibia 1985), exporting nutrients and energy. Even though mortality will thin out the cohort during the estuarine phase, export of energy and nutrients is possible, depending on timing of the migration relative to changes in total cohort biomass. Although several authors have suggested that emigration of fish may export energy from estuaries (Bozeman and Dean 1980; Odum 1980; Weinstein et al. 1980; Wiegert and Pomeroy 1981; Currin et al. 1984; Deegan and Thompson 1985; Zijlstra 1988), there is no direct evidence.

In this paper, I provide evidence that biotic transport by fish can be an important source of energy and nutrients to coastal marine ecosystems. I describe the role of gulf menhaden (*Brevoortia patronus*) migration to the flow of energy, C, N, and P between an estuary and the Gulf of Mexico. I determined the material transported by a cohort of gulf menhaden by measuring timing of migration relative to changes in biomass, body

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energy, and nutrient composition. My hypothesis was that gulf menhaden would export material to the offshore system because they use estuaries as young-of-the-year, a period of their life history when cohort biomass increases due to growth should exceed losses due to mortality (Tyler and Gallucci 1980).

Natural History and Ecological Background

Gulf menhaden have a life-history pattern with many characteristics that indicate that they may transport energy or nutrients across the coastal boundary. Young-of-the-year gulf menhaden are ubiquitous and dominant (30–50% by number) members of Gulf of Mexico estuarine communities from the Yucatan Peninsula, Mexico, to Tampa Bay, Florida (Christmas et al. 1982; Lassuy 1983; Deegan and Thompson 1985). Spawning occurs in offshore waters during the winter and peaks in December and January (Lassuy 1983). Eggs hatch at sea and ocean currents carry the larvae into estuaries where they develop into juveniles (Lassuy 1983; Deegan 1986). During their stay in estuarine waters, growth is rapid and mortality low (Deegan 1990). The timing of migration is fairly well known, with peak larval immigration in February and juvenile emigration in the fall (Lassuy 1983; Deegan 1990). Adult and juvenile menhaden are phytoplankton and detritus filter feeders (Deegan 1986; Deegan et al. 1990) and are a major food item for offshore predatory fishes. They support one of the largest commercial fisheries in North America (Vaughn 1987) which depends primarily on age 1 (80% of landings) taken directly offshore from the nursery areas (Nicholson 1978; Vaughn 1987).

I studied this process in Fourleague Bay, Louisiana (29°15′N, 91°15′W), an estuary of the north-central Gulf of Mexico. It is shallow (average depth about 1 m) with approximately 10 000 ha of open bay and 50 000 ha of brackish to salt marsh. Marsh vegetation consists of *Juncus roemerianus*, *Spartina patens*, *Spartina alterniflora*, *Distichilis spicata*, *Scirpus olneyi*, and *Sagittaria lancifolia*. The Atchafalaya River is the major source of freshwater and maintains a salinity gradient from fresh at the northern end of the bay to brackish at Oyster Bayou.

Methods

Field Sampling

The estuarine residence period and the size of immigrating larvae and emigrating juveniles were determined by sampling in the estuary and the tidal pass. Paired stationary wing nets (3 × 3 m, cod-end mesh 1.6 mm) were fished at the surface in the tidal pass during the first week of February, March, April, September, October, and December 1982 and February 1983. Duplicate samples were taken every 3 h for 52 h each sampling period for a total of 34 samples per month. Gulf menhaden were sampled monthly from July 1981 to December 1983 from estuarine open bay habitats (four stations) using replicate 10-min otter trawl tows (4.9 m headrope, 19.1-mm-mesh wings, and 4.8-mm-mesh cod end) that covered approximately 0.35 ha per tow (Deegan 1990).

Laboratory Analysis

Total number of gulf menhaden per sample was counted and individual wet mass (0.01 g) and standard length (millimetres) measured. N, P, C, energy composition, and dry mass (0.001 g) were determined on frozen fish. Energy composition was calculated from protein (6.25 × Kjeldahl N) and lipid (hexane extraction) percent composition using an energy equivalent of 40.16 $kJ \cdot g^{-1}$ for lipid and 23.64 $kJ \cdot g^{-1}$ for protein (Brett and Groves 1979; Gardiner and Geddes 1980). N and P were determined on a Technicon autoanalyzer after micro-Kjeldahl and nitric acid/perchloric acid digestion, respectively, using approximately 100 mg of sample. Samples were stored under desiccation; before weighing, they were dried for 1 h at 75°C. C was estimated as a percentage of dry mass based on the relationship between C percent composition and caloric content (Durbin et al. 1983). Differences in size (millimetres or grams dry weight), nutrient (grams per gram dry weight or percentage), and energy (kilojoules per gram) composition of larvae and juveniles were tested using *t*-tests (Steel and Torrie 1980).

Estimation of Net Flux

To estimate the importance of gulf menhaden migration to estuarine nutrient and energy flows, I did a mass balance calculation for two separate year-classes (cohorts) for the duration of their time in the estuary. I estimated the net nutrient and energy fluxes by gulf menhaden on a per fish (capita) basis and the areal net flux by the entire cohort on a per square metre of marsh basis.

Net flux per fish per year was estimated by adjusting the mass of one juvenile emigrating by the mass of larvae which immigrated, after accounting for differences in nutrient composition between larvae and juveniles. This is a mass balance based on the relative number of fish immigrating and emigrating using weight and energy and nutrient contents of fish at migration. The number of immigrating larvae (N_1) required to balance one juvenile emigrating was estimated from the relative dry weights and energy and nutrient contents of fish captured in the tidal pass during migration (Equation 1, Table 1). The number of these larvae which emigrated as juveniles (N_j) was calculated by applying a mortality rate (Z, Deegan 1990) over the time period (t) spent in the estuary (Equation 2, Table 1). The net biomass transported per fish (F_f; Equation 5, Table 1) was calculated by subtracting the biomass of immigrating larvae (B_i; Equation 3, Table 1) from the biomass of emigrating juveniles (B_j; Equation 4, Table 1) on a per fish basis.

Areal net flux (F_m, grams or kilojoules per square metre per year) was estimated by multiplying net flux per fish (F_f) emigrating by the total number of juveniles in the cohort (C) which left the estuary and then dividing by estuarine marsh area (A) (Equation 6, Table 1). The number of emigrating juveniles in the 1982 and 1983 year-classes was estimated at 53.7×10^9 and 85.3×10^9 individuals, respectively (Nelson and Ahrenholtz 1986; Vaughn 1987). The area of salt and brackish marsh was 4.99×10^9 m^2 (Chabreck 1972).

Because the estimates of flux are based on a series of measurements, all of which have variation associated with them, I estimated net flux separately for the 1982 and 1983 year-classes. This gives separate estimates which indicate the degree of variation in transport by fish that might occur from year to year.

Results

Migration Pattern and Size at Migration

Each year-class occupied the estuary from February to November–December of a single year or about 270 d (Fig. 1 and 2). Peak abundance of larvae in both the tidal pass and in the main open water of the estuary occurred in February in both years (Fig. 1A). Peak migration of juveniles out of the estuary was in late November or early December, as indicated by

TABLE 1. Equations used to estimate net flux of biomass, energy, and nutrients. See Estimation of Net Flux in the Methods section for a description.

	Equation	Parameters
(1)	$N_1 = (W_t C_t)/(W_0 C_0)$	N_1 = Number of immigrating larvae needed to balance one juvenile emigrating W_t = Dry mass (g) of one juvenile emigrating (inside estuary and tidal pass study, Table 2) W_0 = Dry mass (g) of one larvae immigrating (inside estuary and tidal pass study, Table 2) C_t = Energy ($kJ \cdot g^{-1}$) or nutrient ($g \cdot g^{-1}$) content of a juvenile at emigration (inside estuary and tidal pass study, Table 2) C_0 = Energy ($kJ \cdot g^{-1}$) or nutrient ($g \cdot g^{-1}$) content of larvae at immigration (inside estuary and tidal pass study, Table 2)
(2)	$N_j = N_i e^{-Zt}$	N_j = Number of larvae that survive to emigrate as juveniles Z = Instantaneous mortality rate (Deegan 1990) t = Duration of stay in estuary (inside estuary and tidal pass study, Fig. 1 and 2 and Deegan 1990)
(3)	$B_i = N_i W_0 C_0$	B_i = Biomass of larvae immigrating, adjusted for nutrient or energy content
(4)	$B_j = N_j W_t C_t$	B_j = Biomass of juveniles emigrating, adjusted for nutrient or energy content
(5)	$F_f = (B_j - B_i)/N_j$	F_f = Net flux per juvenile emigrating
(6)	$F_m = (F_f C)/A$	F_m = Net flux by the cohort per square metre of marsh area C = Number of juveniles in year-class (Nelson and Ahrenholtz 1986; Vaughn 1987) A = Area of salt and brackish marsh in estuaries (Chabreck 1972)

declining abundances in the bay and increasing catches in the tidal pass (Fig. 1B). The average size range for both year-classes was 20–22 to 80–110 mm and 0.015–4.0 g dry mass for larvae and juveniles, respectively (Table 2; Fig. 2). There was little difference in size at immigration between year-classes (0.014 versus 0.015 g); however, the 1983 year-class was slightly smaller than the 1982 year-class (3.7 versus 4.1 g dry weight, respectively) at emigration (Table 2) based on samples from the tidal pass. Samples from within the bay (Fig. 2) indicate that a few fast-growing individuals of the 1983 year-class may have emigrated early. There was no significant difference between year-classes in any measure of size at immigration or emigration (*t*-tests, $p > 0.05$). The smallest larval menhaden taken in this study was 18 mm; the largest juvenile was 146 mm.

Nutrient and Energy Composition

Nutrient (percentage) and energy (kilojoules per gram) composition differed between larvae and juveniles (Table 2). Larvae averaged 22.9 $kJ \cdot g^{-1}$, 12.6% N, and 2.2% P, while juveniles averaged 24.1 $kJ \cdot g^{-1}$, 10.1% N, and 2.4% P. The differences in energy and nitrogen composition were significant (*t*-tests) (energy: $t = 6.69$, $p < 0.001$; N: $t = 4.16$, $p < 0.001$). Larvae and juveniles averaged 50 and 56% C, respectively.

Estimates of Net Flux

Net fluxes of energy and all nutrients were out of estuary to offshore for both the 1982 and 1983 cohorts (Table 3). Net flux per capita (fish) per year was estimated at 4.0 and 1.9 g biomass, 95.5 and 47.7 kJ, 2.2 and 1.2 g C, 0.4 and 0.1 g N, and 0.09 and 0.05 g P for the 1982 and 1983 year-classes, respectively. Comparison of the biomass of an individual fish emigrating from the estuary (Table 2) to the biomass exported per fish after adjusting for larval input (Table 3) indicates that the importance of the larval input adjustment varies from year to year and is highly dependent on the mortality rate. Instantaneous mortality rates (per day) were different, averaging 0.007 and 0.018 for the 1982 and 1983 year-classes, respectively (Deegan 1990). For the 1982 cohort, mortality was low and the adjustment for larval input was minor (4.1 g biomass per fish without adjusting versus 4.0 g export biomass per fish after adjusting for larval input). For the 1983 cohort, mortality was higher, and the adjustment for larval input lowered the estimated export by 45% (3.7 g biomass per fish versus 1.9 g export biomass per fish). The flux was 43 and 33 g biomass, 1050 and 811 kJ, 25 and 20 g C, 4.4 and 1.9 g N, and 1 and 0.9 g P for the 1982 and 1983 year-classes, respectively (Table 3). Despite a substantially lower flux per fish rate, the areal flux for the 1983 year-class was close to the areal flux for the 1982 year-class because the number of individuals in the 1983 cohort was large.

Discussion

This study suggests that biotic transport is important in the movement of energy and nutrients across coastal ecosystem boundaries (Table 3). Since the original proposal of the outwelling hypothesis (Odum 1968), considerable effort has been directed at determining kinds and quantities of materials passively transported in water (Nixon 1980; Odum 1980). Although not all estuaries export organic matter, the average passive export of organic matter (particulate and dissolved) is between 100 and 200 $g\,C \cdot m^{-2} \cdot yr^{-1}$ for estuaries on the middle Atlantic and Gulf coasts (Nixon 1980). Happ et al. (1977) estimated that 140–190 $g\,C \cdot m^{-2} \cdot yr^{-1}$ was passively exported to the nearshore coastal zone as particulate or dissolved organic C from Barataria Bay, Louisiana. This C is believed to be an important subsidy to the nearshore coastal zone, perhaps a C

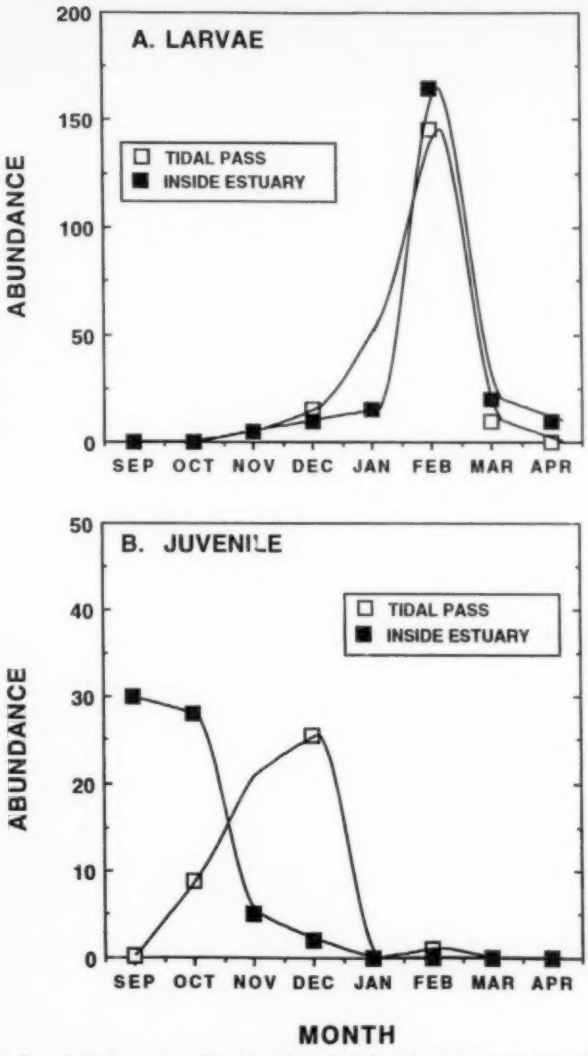

FIG. 1. Emigration and immigration of (A) larval and (B) juvenile gulf menhaden in Fourleague Bay, Louisiana. Abundance in tidal pass is the average number of fish per cubic metre from wing net sampling in Oyster Bayou. Abundance in the estuary is the average number of fish per square metre based on trawl data from open bay stations inside the estuary (Deegan 1990).

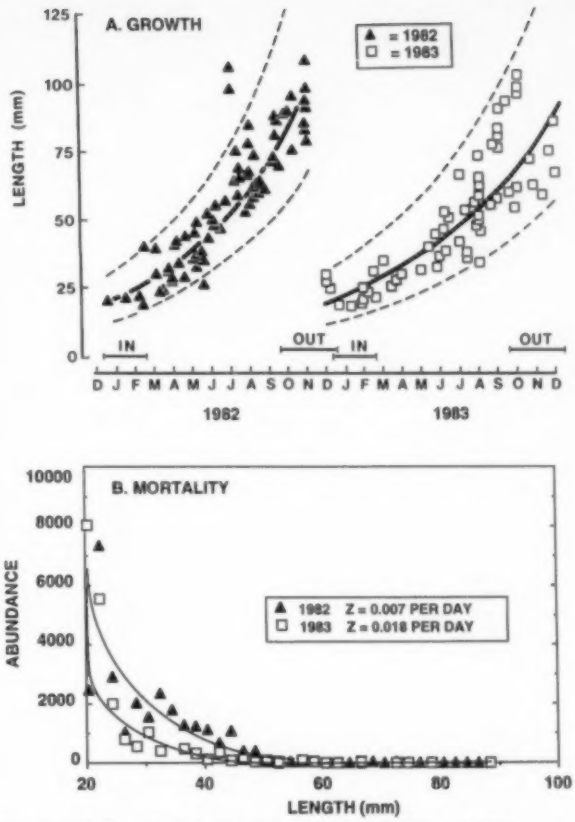

FIG. 2. (A) Growth and (B) mortality for the 1982 and 1983 year-classes of young-of-the-year gulf menhaden in Fourleague Bay, Louisiana (Deegan 1990). Abundances in the bay after November were very low, and the data on length are not plotted. Mortality estimates are based on changes in length frequency and abundance (Deegan 1990). Horizontal lines on the growth graph indicate migration periods (in = immigration, out = emigration). See Table 2 for comparison with data on size at migration based on sampling in the tidal pass.

supplement equal to 25–50% of the offshore production (Nixon 1980; Madden et al. 1988). Happ et al. (1977) did not estimate N or P export for Louisiana. However, Nixon (1980), in his summary, estimated average N and P export at 2–5 g N and 0.4–1.8 g $P \cdot m^{-2} \cdot yr^{-1}$. Estimates of transport by gulf menhaden are much lower for C (22 g $C \cdot m^{-2} \cdot yr^{-1}$) but of the same relative magnitude for N (3.1 g $N \cdot m^{-2} \cdot yr^{-1}$) and P (0.9 g $P \cdot m^{-2} \cdot yr^{-1}$) (Table 3). The C export by gulf menhaden is approximately 5–10% of the primary production of inshore coastal Louisiana (Bahr et al. 1982).

There are important differences between waterborne and fish export in the relative amounts and quality of different nutrients. Nixon (1980) predicted that fish would export a relatively small quantity of C compared with passive waterborne transport of C. This is supported by my results, but because of the lower C:N ratio in gulf menhaden than in detritus, they export greater quantities of N and P relative to C. The C:N ratio, however, is only one indication of the relative quality and usability of organic matter. Gulf menhaden are primarily protein and lipids which are used directly by higher trophic levels in the offshore ecosystem. Unlike fish, exported detritus is of low nutritive value and may be rapidly sedimented or respired by bacteria without entering the higher levels of the offshore food web (Nixon 1980). The export of material in the biomass of gulf menhaden or other species can provide a direct link to higher trophic levels because they are important food items for many of the benthic or pelagic carnivores in the nearshore coastal ecosystem.

All flux estimates integrate several parameters which have natural variation and estimation errors (Nixon 1980). For fish transport, the effect of using a smaller size for larvae, a larger size for juveniles, or shorter estuarine stay would be to increase the net flux out of the estuary (Deegan 1985). The net flux would be zero if the duration of stay were 30 d longer and no further growth occurred. If fish continued to grow at the same rate during a longer residence, then export would increase in

TABLE 2. Average standard length, net weight, dry weight, nutrient content, and energy content of the 1982 and 1983 year-classes of larval and juvenile gulf menhaden during migration in Fourleague Bay, Louisiana (numbers in parentheses are the number of measurements and the standard deviation). See Fig. 2 for comparison with size in the estuary.

	Larvae			Juveniles		
	1982	1983	Average	1982	1983	Average
Length (mm)	22.9 (110, 0.4)	22.4 (230, 0.2)		85.6 (129, 6.5)	80.2 (189, 3.4)	
Wet weight (g)	0.14 (50, 0.02)	0.15 (187, 0.01)		14.6 (78, 3.4)	12.5 (51, 2.6)	
Dry weight (g)	0.015 (45, 0.002)	0.014 (83, 0.001)		4.1 (65, 0.9)	3.7 (28, 0.8)	
Nitrogen (% dry wt.)			12.6 (45, 0.5)			10.1 (52, 0.4)
Phosphorus (% dry wt.)			2.2 (19, 0.5)			2.4 (26, 0.5)
Energy ($kJ \cdot g^{-1}$)			22.9 (45, 0.1)			24.1 (99, 1.1)

TABLE 3. Comparison of biomass, energy, C, N, and P transport by gulf menhaden out of Fourleague Bay, Louisiana, to average quantities of materials passively transported in water for estuaries on the middle Atlantic and Gulf coasts (Nixon 1980).

	Gulf menhaden export			Passive waterborne export
	1982	1983	Average	
Per capita net flux ($fish^{-1} \cdot yr^{-1}$)				
Biomass (g)	4.0	1.9	2.95	
Energy (kJ)	95.5	47.7	71.6	
C (g)	2.2	1.2	1.7	
N (g)	0.4	0.1	0.3	
P (g)	0.09	0.05	0.07	
Areal net flux ($m^{-2} \cdot yr^{-1}$)				
Biomass (g)	43	33	38	
Energy (kJ)	1050	811	930	
C (g)	25	20	22.5	100–200
N (g)	4.4	1.9	3.1	2–5
P (g)	1.0	0.9	0.9	0.4–1.8

proportion to the time spent in the estuary. The pattern and timing of migration of gulf menhaden documented in this study in Fourleague Bay confirm the results of studies in other estuaries (Lassuy 1983). Gulf menhaden immigration peaks in February and March, with the exact timing a function of offshore, wind-driven currents (Lassuy 1983; Deegan 1990). Individual size and timing of emigration are more variable than those of immigration and are determined by productivity in the estuary and the onset of cooler temperatures (Deegan 1990). Cooler temperatures and low productivity in the estuary produce slower-growing fish with a higher mortality rate, as exemplified by the 1983 year-class (Loesch 1976; Lassuy 1983; Deegan and Thompson 1987; Deegan 1990).

Export of biomass by young-of-the-year gulf menhaden is consistent with estimates of cohort biomass derived from independent information from the offshore fishery. Growth and mortality estimates from the fishery indicate that peak cohort biomass is reached when the fish are 1–1.5 yr old (Vaughn 1987), or about 3–6 mo after they leave estuarine areas.

One of the original paradigms of estuarine function was that estuaries produce annual excesses of plant organic matter, some of which is exported seaward and supports coastal fisheries (Odum 1968). My results indicate that estuaries support coastal fisheries by direct export of fish biomass, which is a major link in the coastal food web and, in some species, forms the basis for commercial or recreational fisheries.

Many other commercial and recreationally important species have the potential to transport nutrients and energy. The young-of-the-year of shrimp, crabs, Atlantic croaker (*Micropogonias undulatus*), spot (*Leiostomus xanthurus*) and other species also have high growth and low mortality in estuarine systems and a similar migration pattern (Yakupzack et al. 1977; Weinstein and Walters 1981; Currin et al. 1984). The amount transported by each of these species is probably less than my estimate for gulf menhaden because the number of individuals in a cohort is lower. The cumulative transport, however, of 6–10 additional species may be significant.

This study underlines the importance of understanding the life-history patterns and population dynamics of animal species in assessing their effects on the ecosystem processes of nutrient cycles and food web dynamics. In coastal waters, the same fish that export energy and nutrients to nearshore coastal ecosystems and are a major forage fish for higher trophic levels also form the basis for many commercial and recreational fisheries. We need to consider carefully what impact the harvesting of nutrient exports in a fishery may have on the food webs and overall productivity of nearshore coastal ecosystems. Nutrient additions to ecosystems may replace dissolved nutrient flows, but do not substitute for fish that directly feed higher trophic levels in coastal marine systems.

Acknowledgments

This study was funded by Sea Grant, Sigma Xi, the Coastal Fisheries Program, and the Department of Marine Sciences at Louisiana State University. This paper was substantially improved by the comments of J. Day, B. Thompson, C. Neill, J. Hobbie, B. Peterson, J. Goering, and anonymous reviewers.

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Mitochondrial DNA Variation in Striped Bass (*Morone saxatilis*) from Canadian Rivers

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Wirgin, I. I., T.-L. Ong, L. Maceda, J. R. Waldman, D. Moore, and S. Courtenay. 1993. Mitochondrial DNA variation in striped bass (*Morone saxatilis*) from Canadian rivers. Can. J. Fish. Aquat. Sci. 50: 80–87.

Mitochondrial DNA (mtDNA) was analyzed to determine the genetic relatedness of striped bass (*Morone saxatilis*) populations in tributaries to the Gulf of St. Lawrence and the Bay of Funday. Mitochondrial DNA genotype frequencies were determined for striped bass from the Shubenacadie River (Bay of Fundy) and the Miramichi and Tabusintac rivers (Gulf of St. Lawrence). These mtDNA genotype frequencies were compared with those of striped bass representative of the Atlantic coastal migratory stock originating in the Hudson River and Chesapeake Bay. Differences in the frequencies of mtDNA length variants permitted discrimination of the Shubenacadie River from the Miramichi River and Tabusintac River populations and all three Canadian populations from the U.S. spawned coastal migratory stock. No difference in the frequency of mtDNA length variants was observed between Tabusintac River and Miramichi River striped bass. Heteroplasmy for mtDNA length variants was observed in 35% of Gulf of St. Lawrence fish, the highest frequency observed in any striped bass population. These results highlight the genetic heterogeneity of these Canadian striped bass populations and their distinctiveness from U.S. stocks. Future efforts to restore these depleted Canadian striped bass populations should consider the impact of stock transfer on endemic striped bass gene pools.

L'ADN mitochondrial (ADNmt) a été analysé afin de déterminer la parenté génétique des populations de bar rayé (*Morone saxatilis*) trouvé dans les tributaires du golfe du Saint-Laurent et le baie de Fundy. Les fréquences génotypiques d'ADNmt ont été déterminées chez le bar rayé capturé dans la rivière Shubenacadie (baie de Fundy) ainsi que dans les rivières Miramichi et Tabusintac (golfe du Saint-Laurent). Ces fréquences génotypiques d'ADNmt ont été comparées à celles de bars rayés représentatifs du stock migratoire de la côte Atlantique qui provient de la rivière Hudson et de la baie Chesapeake. Des différences dans les fréquences des variants en longueur d'ADNmt ont permis de faire une distinction entre la population de la rivière Shubenacadie et les populations des rivières Miramichi et Tabusintac, ainsi qu'entre les trois populations canadiennes et le stock migrateur côtier des États-Unis. On n'a pas observé de différences dans la fréquence des variants en longueur l'ADNmt entre les bars rayés de la rivière Tabusintac et ceux de la rivière Miramichi. L'hétéroplasmie au niveau des variants en longueur de l'ADNmt a été observée chez 35% des sujets du golfe du Saint-Laurent; il s'agit de la plus haute fréquence observée dans n'importe quelle population de bars rayés. Les résultats montrent l'hétérogénéité génétique de ces populations canadiennes et montrent aussi qu'elles diffèrent des stocks des États-Unis. Dans toute tentative éventuelle de rétablissement de ces populations canadiennes en déclin, il y a lieu de considérer les répercussions des transferts de stocks sur les pools génétiques des bars rayés endémiques.

Received February 13, 1992
Accepted July 9, 1992
(JB409)

Reçu le 13 fevrier 1992
Accepté le 9 juillet 1992

Striped bass (*Morone saxatilis*) is an anadromous species which supports important commercial and recreational fisheries throughout much of its North American distribution. Historically, the Atlantic coast range of striped bass extended from the St. Lawrence River, Canada, to the St. Johns River, Florida (Raney 1952). Small populations were also found in many of the major drainages of the Gulf of Mexico extending from northwest Florida to Texas (Matlock et al. 1984). In the past century, striped bass were introduced into the Pacific Ocean and in many freshwater impoundments throughout much of North America (Westin and Rogers 1978). The Hudson River and tributaries of the Chesapeake Bay support the largest striped bass spawning stocks (Boreman and Austin 1985), and a portion of these stocks migrates along the Atlantic coast within a range that extends from northern North Carolina to the Bay of Fundy (Nichols and Miller 1967; Waldman et al. 1990). However, striped bass spawned at the southern (Dudley et al. 1977) or northern (Magnin and Beaulieu 1967) extreme of their range are believed to be nonmigratory, and thus occur within their natal river systems and proximal waters throughout their life cycle.

Stiped bass have become less abundant in many Canadian rivers in recent decades (Williamson 1974; Rulifson et al. 1987). Adult striped sea bass are currently found in many rivers

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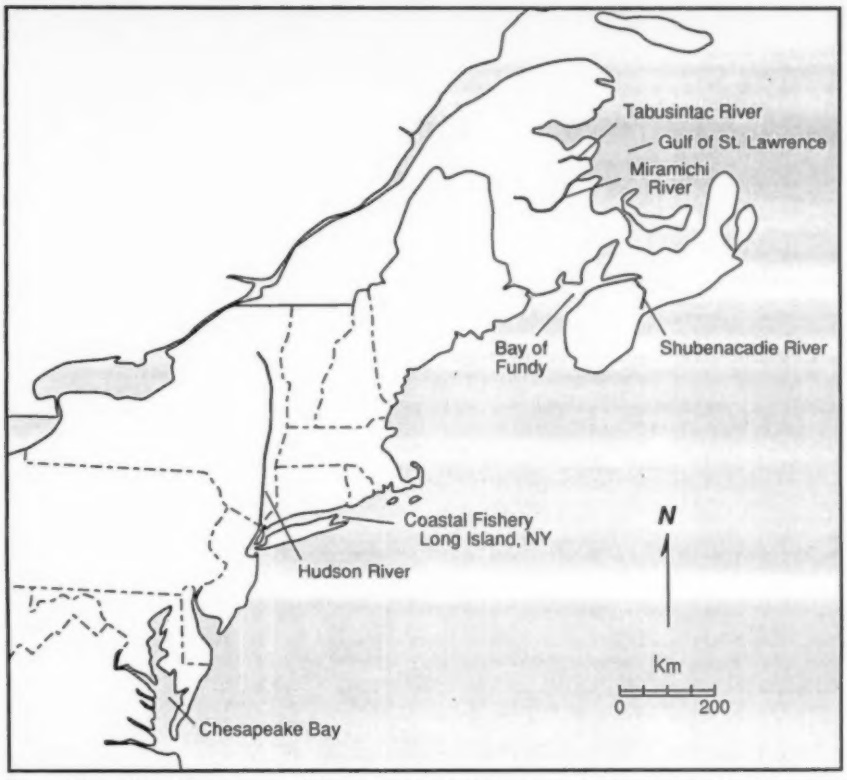

FIG. 1. Map of the Atlantic coast of North America depicting the collection sites of striped bass used in this study.

entering the Bay of Fundy, including the Shubenacadie and the Annapolis rivers, Nova Scotia, and the St. John and Petitcodiac rivers, New Brunswick (Scott and Scott 1988). Since the 1870s, commercial landings of striped bass in the Bay of Fundy counties of Nova Scotia have fluctuated between 0.5 and 13 metric tons (t) and in New Brunswick counties between <4.5 and 55 t (Jessop 1991). In recent years, evidence of successful spawning activity within the Bay of Fundy watershed, demonstrated by the presence of young-of-the-year and yearling fish, has only been observed in the Shubenacadie River (Rulifson et al. 1987). Although some striped bass eggs have been collected in the Annapolis and St. John rivers, juvenile life stages have been either very rare or absent (Williamson 1974; Williams et al. 1984; M. Dadswell, pers. comm.). In contrast, juvenile striped bass have been reported in many rivers along the Gulf of St. Lawrence (Hogans and Melvin 1984), and commercial harvests of striped bass have ranged from 2 to 50 t annually in southern Gulf rivers (Miramichi, Tabusintac, Kouchibouguac, Buctouche, and Richibucto) during the 1980s (LeBlanc and Chaput 1991). Recent observations suggest extirpation of the St. Lawrence River striped bass population (J. Dodson, Laval University, pers. comm.; Melvin 1978). Recaptures of Hudson River and Chesapeake Bay tagged striped bass within several rivers of the Bay of Fundy (Waldman et al. 1990; R. Dorazio, U.S. Fish and Wildlife Service (USFWS), pers. comm.) demonstrate entrance of migratory U.S. stocks into these Canadian systems. To date, Hudson River or Chesapeake Bay tagged striped bass have not been recaptured within the Gulf of St. Lawrence or its rivers (Waldman et al. 1990; R. Dorazio, USFWS, pers. comm.).

Restoration of depleted striped bass populations within rivers along both the Gulf of St. Lawrence and the Bay of Fundy has been proposed using hatchery-reared fish produced from broodstock collected from Canadian rivers. Characterization of genetic variation and formulation of management policies to preserve or restore genetic diversity are recognized goals of successful fisheries management (Nelson and Soulé 1987). To date, there is little information on genetic relationships among Canadian striped bass populations or between Canadian and U.S. striped bass populations. Williamson (1974) compared meristic characters between striped bass from the Annapolis and St. John rivers and found no significant differences between the two systems. Melvin (1978) studied electrophoretic variation in 48 protein bands and did not find sufficient variability to aid in distinguishing striped bass collected in the St. John River, Shubenacadie Lake, and Tabusintac River.

Much effort has been directed toward the identification of striped bass stocks in the United States (for a review see Waldman et al. 1988). Among genetic approaches, analysis of allozyme variability revealed extremely low levels of polymorphism (Otto 1975; Grove et al. 1976; Sidell et al. 1980; Wirgin 1987) and therefore precluded its use in population studies. We previously used restriction fragment length polymorphism analysis (RFLP) of mitochondrial DNA (mtDNA) to differentiate between Hudson River and Chesapeake Bay stocks (Wirgin et al. 1990b) and to discriminate between Gulf of Mexico and southern Atlantic striped bass populations (Wirgin et al. 1989). Furthermore, Chapman (1987, 1989, 1990) used RFLP analysis of mtDNA to define striped bass poplations within the Chesa-

peake Bay. In summary, these studies revealed unusually low levels of mtDNA diversity in striped bass in comparison with other fishes (maximum nucleotide diversity between two individuals was $p = 0.0004$); however, variation in the length of the mtDNA molecule and two mtDNA restriction site polymorphisms did permit discrimination among some striped bass stocks. Mitochondrial DNA length divergence among the mitochondria from a single individual (length heteroplasmy) was observed in all striped bass populations (Wirgin et al. 1989, 1990b). RFLP analysis of repetitive (Wirgin et al. 1991) and single-copy nuclear DNA sequences has also been used to define certain striped bass populations (Wirgin and Maceda 1991).

In this study, we used RFLP analysis of mtDNA to determine the genetic relatedness of two striped bass populations within the Gulf of St. Lawrence (Gulf) and between these two Gulf populations and striped bass within the Shubenacadie River in the Bay of Fundy. We also compared the mtDNA genotype frequencies in these Canadian rivers with those observed in striped bass collected from the Hudson River, several spawning rivers in the Chesapeake Bay, and from the Atlantic coastal migratory fishery (Wirgin et al. 1990b; I. I. Wirgin, unpubl. data). This analysis permitted an estimation of the genetic relatedness of the Shubenacadie River and Gulf populations to migratory U.S. stocks.

Materials and Methods

Striped bass were collected during 1990 and 1991 from three Canadian rivers: 49 fish from the Tabusintac, 53 from the Miramichi, and 34 from the Shubenacadie (Fig. 1, and See Table 1 for sample characterizations). We considered these fish to have originated in the rivers in which they were collected because all of the fish were of premigratory age classes, juveniles and yearlings, based on their length range (Table 1). Age-0 and age-1 striped bass from the two major migratory mid-Atlantic stocks, those of the Chesapeake Bay and the Hudson River, do not make long-distance migrations (Kohlenstein 1981; Waldman et al. 1990). Less is known about the movements of Canadian striped bass. Rulifson et al. (1987) did observe juvenile striped bass in the northern Bay of Fundy that probably originated from the Shubenacadie–Stewiacke system.

Mitochondrial DNA genotypes of these Canadian striped bass samples were compared with mtDNA genotypes in U.S. populations (Wirgin et al. 1990b; I. I. Wirgin, unpubl. data). Striped bass collected from the Hudson River and Chesapeake Bay spawning grounds during spawning season were assumed to represent each of those stocks (Waldman et al. 1988). Samples from Long Island, New York, were further assumed to include Chesapeake Bay and Hudson River striped bass, in unknown proportions (Van Winkle et al. 1988).

Mitochondrial DNA fragments were visualized by Southern blot analyses using the highly purified mtDNA (Wirgin 1987) from a single striped bass liver as a probe (Wirgin et al. 1990b). Total DNA was isolated for analysis as described in Wirgin et al. (1990a) using the technique originally reported by Saghai-Maroof et al. (1984). DNA from all samples was digested with four restriction enzymes, *Acc* I, *Dra* I, *Hind* III, and *Rsa* I, which provided clear resolution of mtDNA length variants. Twenty samples from each river were further digested with a battery of 12 more restriction enzymes including *Apa* I, *Ava* II, *Bgl* I, *Eco* RV, *Hinf* I, *Hae* II, *Ksp* I, *Msp* I, *Nae* I, *Sac* I, *Taq* I, and *Xba* I. These enzymes were selected to include all those which have shown restriction site polymorphisms in striped bass from other spawning systems and several enzymes which had not previously been tested on striped bass mtDNA.

Digested DNAs were separated in 1.0–1.6% horizontal agarose gels and DNA fragments were Southern blotted (Southern 1975) onto ZetaBind membranes (Cuno, Meriden, CT). Approximately 5–50 ng of striped bass mtDNA probe was ^{32}P radiolabeled by nick-translation (Rigby et al. 1977). Filters were prehybridized and hybridized at 65°C as described by Wahl et al. (1979) using dried milk as a blocking agent (Wirgin et al. 1990a). Final wash conditions were 0.1 × SSC/0.1% SDS at 65°C for 30 min. Dried filters were exposed to Kodak XAR 5 film at −80°C for 1–5 d with intensifying screens. Molecular weights were determined by comparisons with DNA standards. Frequencies of polymorphic fragments were determined for each restriction enzyme in each population and composite genotypes for each fish were compiled based on the data from all individual restriction enzyme digests. Chi-square analysis (Statview, Abacus Concepts, Inc.) was used to determine the significance of frequency differences of mtDNA length variants in striped bass from the different collection sites.

Results

Mitochondrial DNA isolated from juvenile striped bass from three Canadian rivers was screened for polymorphisms with 4–16 different restriction enzymes and these mtDNA genotypes were compared with those detected in earlier studies from striped bass collected from U.S. spawning systems and coastal sites (Wirgin et al. 1990b; I. I. Wirgin, unpubl. data). With the exception of *Hae* II and *Xba* I, all restriction enzymes revealed polymorphisms in the length of the mtDNA molecule (mtDNA length variants). For each restriction enzyme used, a single mtDNA fragment was diagnostic in identifying the mtDNA length genotype (Fig. 2). The various mtDNA length genotypes differed by increments of about 100 base pair (bp) in molecular size. In total, four different mtDNA length genotypes were detected in striped bass from these three Canadian populations:

TABLE 1. Characterization of Canadian striped bass samples.

Collection site	*N*	Collection date	Gear	Mean fork length (SE) and range (cm)
Tabusintac River	49	Nov. 2, 1990	Smelt bag net	14.7 (0.10) 13.5–16.2
Miramichi River	33	Oct. 18, 1990	Smelt bag net	16.9 (0.85) 14.0–29.2
Miramichi River	20	Oct. 22, 1990	Smelt bag net	15.3 (0.11) 14.7–16.7
Shubenacadie River	34	Sept. 6, 1991	Beach seine	10.5 (0.95) 7.2–23.0

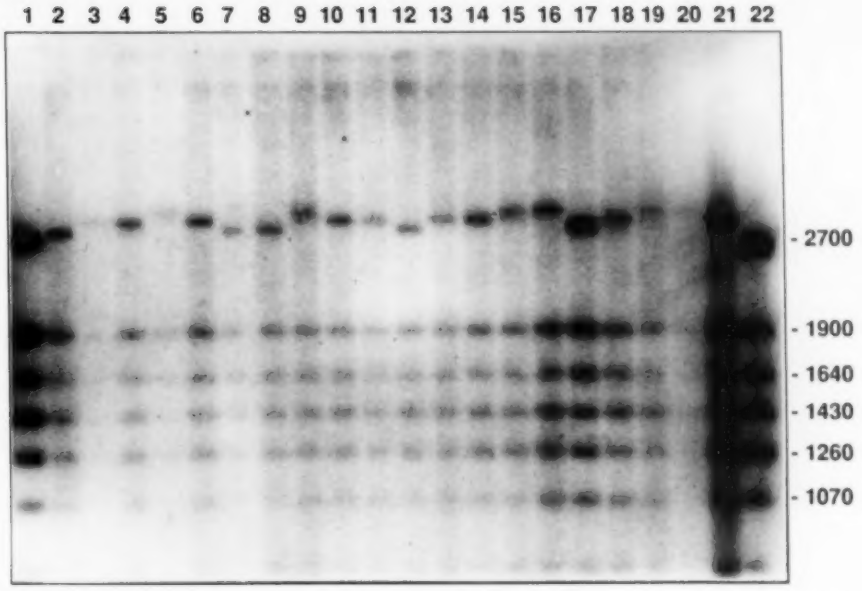

FIG. 2. Shubenacadie striped bass total DNA digested with *Hinf* I and hybridized to a ^{32}P-labelled striped bass mtDNA probe. Variation can be seen in the size of the largest mtDNA fragment in each lane. Lane to mtDNA length genotypes designations are as follows: A^1 — 5, 9, 15, 16, 19, 20; A^2 — 3, 4, 6, 10, 11, 13,1 4, 18, 21; B — 2, 7, 8, 12, 17. Striped bass mtDNA standards are in lanes 1 (genotype C) and 22 (genotype D) (C)).

A^1 (18 100 bp), A^2 (18 000 bp), B (17 775 bp), and C (17 635 bp). Representative standard deviation values for a 2815-bp fragment were ±22 bp. In comparison, a total of seven different mtDNA length genotypes have previously been detected in U.S. populations including all four genotypes detected in these Canadian samples (Wirgin et al. 1989, 1990b).

The frequencies of mtDNA length genotypes in the various populations are depicted in Table 2. Striped bass from the Miramichi and Tabusintac rivers revealed three mtDNA length genotypes, A^2, B, and C, whereas four length genotypes, A^1, A^2, B, and C, were observed in striped bass from the Shubenacadie River. Given the rarity of both A genotypes in Gulf and U.S. east coast populations, frequencies of these genotypes were pooled as a single A genotype in subsequent comparisons.

The D length genotype commonly seen in striped bass from all U.S. spawning populations and at a relatively high frequency (>25%) was not detected in mtDNA from these Canadian striped bass. The C length genotype, the most common mtDNA genotype in all U.S. striped bass spawning populations (>40%), was detected only in a single striped bass from the Shubenacadie River. In comparison, the C length genotype was the second most frequent in the total Gulf of St. Lawrence sample. Additionally, 65% of striped bass from the Shubenacadie River displayed an A length genotype, which is evident in less

TABLE 2. Frequencies of mtDNA length variants and heteroplasmy in striped bass populations.

Collection site (*N*)	mtDNA length genotypes				% heteroplasmic
	A	B	C	D	
Miramichi River (53)	0	30	23	0	38
Tabusintac River (49)	1	23	25	0	35
Shubenacadie River (34)	22	11	1	0	12
Hudson River (78)[a]	2	28	26	22	17
Hudson River (131)[b]	5	40	58	28	16
Hudson River (92)[b]	2	21	41	28	—
Chesapeake Bay (61)[a]	1	2	36	22	16
Chesapeake Bay (188)[b]	3	18	100	67	12
Long Island, New York (24)[a,c]	0	5	17	2	33
Long Island, New York (111)[b,d]	4	25	48	34	—

[a]Wirgin et al. 1990b.
[b]I. I. Wirgin et al., unpubl. data.
[c]Montauk, Long Island, New York, fall 1983.
[d]East Hampton, Long Island, New York, fall 1989.

than 2% of striped bass representative of the U.S. coastal migratory stock and whose frequency does not exceed 5% in any U.S. striped bass spawning population, including those at the southern extreme of the species' range. Surprisingly, the A genotype was seen only in one striped bass from the Miramichi or Tabusintac River.

The genetic distinctiveness of these striped bass populations was evaluated based on frequency differences in these mtDNA length variants. Striped bass in the two rivers in the Gulf of St. Lawrence, the Miramichi and Tabusintac, exhibited similar frequencies of mtDNA length genotypes and therefore could not be distinguished. In contrast, considered individually or in combination, striped bass from both Gulf rivers could be differentiated from the Shubenacadie River collection (Miramichi: $\chi^2 = 47.1, p < 0.0001$; Tabusintac: $\chi^2 = 43.1, p < 0.0001$). Similarly, the two Gulf stocks were highly differentiated from the combined U.S. coastal migratory stock ($\chi^2 = 66.1$, $p < 0.0001$) as was the Shubenacadie River collection ($\chi^2 = 248.3$, $p < 0.0001$). Furthermore, all Canadian stocks were genetically differentiated from all individual populations contributing to the U.S. coastal migratory stock and the combined frequencies for all representatives of the U.S. migratory stock.

Heteroplasmy for mtDNA length variants was observed in a high proportion of Canadian striped bass. Frequencies of heteroplasmic fish were determined for each of three Canadian populations and compared with those previously reported for U.S. spawning stocks (Table 2). Approximately 35% of striped bass collected in the Miramichi and the Tabusintac rivers exhibited heteroplasmy for mtDNA length variants. A much lower frequency (12%) of heteroplasmy was observed in striped bass collected from the Shubenacadie River.

Discussion

Effective management of Canadian striped bass demands a detailed knowledge of stock differentiation within Canadian waters, yet no study has successfully determined the genetic relatedness of striped bass populations in Canadian rivers or the extent of genetic similarity between these populations and spawning stocks of striped bass in the United States. Analysis of mtDNA in striped bass from two populations in the Gulf of St. Lawrence watershed, the Miramichi and Tabusintac rivers, showed high similarity between them in their frequencies of mtDNA length genotypes. These results suggest that observations of mixing of striped bass populations within the Gulf of St. Lawrence (e.g. Bigelow and Schroeder 1953) extend to sufficient reproduction between members of these populations to preclude the evolution of interpopulational genetic heterogeneity. Supporting evidence comes from mark–recapture studies of adult striped bass (3–6 yr old) tagged in the Kouchibouguac River which resulted in many returns from the Miramichi Bay area and lesser numbers from the Tabusintac River. In contrast, juvenile striped bass tagged in the Kouchibouguac River were not recovered outside their natal river (Hogans and Melvin 1984). The lack of significant differences in mtDNA length genotypes between the Miramichi and Tabusintac populations does not necessarily demonstrate that they form a single gene pool. It is possible that these populations are discrete but (1) mix together at an early age, (2) are too recent to have developed significant differences in mtDNA, (3) that recent extirpations have occurred in either of the rivers with repopulation from the other river, or (4) that they acquired or maintained similar mtDNA length genotype frequencies simply due to chance. A more complete understanding of the relationships between these populations and other Gulf populations could be gained by sampling in additional Gulf rivers and by applying more sensitive genetic markers, such as RFLP analysis of nuclear DNA (Wirgin and Maceda 1991).

The highly significant difference in the frequencies of mtDNA length genotypes beween the two Gulf populations and the Shubenacadie River population indicates reproductive isolation of striped bass from these systems. Approximately 65% of Shubenacadie River striped bass displayed the mtDNA A length genotype whereas 1% of striped bass from the two Gulf rivers exhibited this genotype. Genetic differentiation between these populations is supported by the results from limited mark–recapture studies. For example, Rulifson et al. (1987) tagged about 1400 striped bass in the upper Bay of Fundy and of 237 recoveries, none were returned from the Gulf of St. Lawrence. Similarly, Hogans and Melvin (1984) tagged several hundred adult striped bass in the Kouchibouguac River and no recaptures were reported from the Bay of Fundy or its rivers. Absence of striped bass migration between the Bay of Fundy and the Gulf of St. Lawrence may result from prevailing cold water temperatures along the Atlantic coast of Nova Scotia. An area of upwelling restricts temperatures to no more than about 10°C in summer (Scott and Scott 1988), well below the preferred temperature of 18–22°C for adult striped bass (Coutant 1985).

The mtDNA length genotype frequencies of both the Gulf of St. Lawrence and Shubenacadie River samples differed significantly from the frequencies reported for the Hudson River and Chesapeake Bay stocks, or for the pooled Hudson River, Chesapeake Bay, and Long Island, New York, collections. Three mtDNA length genotypes, B, C, and D, are commonly found in the Hudson River and Chesapeake Bay stocks. Frequency differences in these three mtDNA length genotypes are statistically significant both between the Hudson River and Chesapeake Bay stocks (Wirgin 1987; Wirgin et al. 1990b; I. I. Wirgin, unpubl. data) and among some of the populations that comprise the Chesapeake Bay stock (Chapman 1987, 1989, 1990; Wirgin et al. 1990b). Although other mtDNA length genotypes occur in migratory (Wirgin et al. 1990b; I. I. Wirgin, unpubl. data) and nonmigratory (Wirgin et al. 1989) U.S. populations of striped bass, their frequencies were always less than 5%. In contrast, none of the Canadian striped bass samples displayed the D genotype, the frequency of which exceeds 25% in all U.S. populations. Furthermore, only 1 striped bass from the Shubenacadie River exhibited the C genotype, which is the most common in all U.S. spawning systems. Finally, 65% of the Shubenacadie River sample exhibited the A genotype, a mtDNA length genotype previously observed in 1% of migratory mid-Atlantic striped bass and no more than 5% in other U.S. populations (Wirgin et al. 1990b; I. I. Wirgin, unpubl. data).

Base substitutions were extremely rare in striped bass mtDNA from these populations. Only a single base substitution, at an *Acc* I recognition site, was observed. The lack of geographically partitioned differentiation in the distribution of this polymorphism precluded its use in discriminating populations. Despite the use of up to 27 restriction enzymes on mtDNA from striped bass from selected populations representing the species' entire range, only four restriction site polymorphisms (*Acc* I, *Rsa* I, *Taq* I, and *Xba* I) were observed in significant frequencies in any population (Wirgin et al. 1989, 1990b; I. I. Wirgin, unpubl. data). A single striped bass collected from the Delaware River exhibited a base substitution with *Dra* I (I. I. Wirgin and J. R. Waldman, unpubl. data). Striped bass exhibit the lowest of overall mtDNA polymorphism seen in any fish

species ($p \leq 0.0004$) (Wirgin et al. 1989; Billington and Hebert 1991). With the exception of the *Acc* I site polymorphism, all significant restriction site polymorphisms were seen in striped bass from southern populations. Given the likely extirpation of Canadian striped bass populations during the Pleistocene, and their likely recolonization approximately 10 000 yr ago, low levels of mtDNA polymorphisms within and between Canadian populations are to be anticipated.

Heteroplasmy is the phenomenon whereby an individual fish exhibits more than one form of the mtDNA molecule. The frequency of occurrence of heteroplasmy in poikilotherms is higher than in mammals, and most cases of heteroplasmy reported in fish, including striped bass, have represented intraindividual variation in the size of the mtDNA molecule (Billington and Hebert 1991). For example, heteroplasmy for mtDNA length variants has been reported in American shad (*Alosa sapidissima*) (Bentzen et al. 1988; Nolan et al. 1991), bowfin (*Amia calva*) (Bermingham et al. 1986), and white sturgeon (*Acipenser transmontanus*) (Buroker et al. 1990). We observed differences among striped bass populations in the frequency of heteroplasmy. With the exclusion of the Shubenacadie population, a cline was observed in the frequency of heteroplasmy in striped bass populations, such that the incidence was lowest in the southeastern U.S. stocks amd highest in the Miramichi and Tabusintac River populations. Frequencies of heteroplasmic individuals ranged from 5% in the Apalachicola River system draining to the Gulf of Mexico (Wirgin et al. 1989) to 4% in the Roanoke River, North Carolina (I. I. Wirgin et al., unpubl. data), to 20% in the Hudson and Chesapeake spawning systems (Wirgin 1987; Wirgin et al. 1990b) to 35–38% in the Miramichi-Tabusintac collection. In contrast, only 12% of striped bass collected in the Shubenacadie River were heteroplamsic, providing further evidence of a lack of exchange between striped bass spawned in the Gulf of St. Lawrence and the Bay of Fundy. Similar to our earlier studies, several samples exhibited more than one heteroplasmic mtDNA length genotype, up to a maximum of four different-sized mtDNA molecules in a single fish. Additionally, no relationship was seen between the size of the predominant mtDNA length genotype and the size of the heteroplasmic length genotypes. For example, it was equally likely that a fish with a predominant B mtDNA length genotype was heteroplasmic for the A or C mtDNA length genotype. Heteroplasmy for common length genotypes such as B and C was observed in these three Canadian populations. This observation is consistent with our earlier reports (Wirgin et al. 1989, 1990b; I. I. Wirgin et al., manuscript submitted), yet contrasts with Chapman (1990) who only reported the presence of heteroplasmy for rarer mtDNA genotypes in striped bass collected from Chesapeake Bay.

Our results show that these three Canadian populations of striped bass are strongly differentiated genetically from extant U.S. populations. However, the degree to which the Canadian samples differed from U.S. stocks was surprising, given that the Canadian populations were almost certainly derived via post-Pleistocene dispersal from U.S. populations. Coastal Nova Scotia and New Brunswick were glaciated until approximately 13 000 yr ago (Underhill 1986), and their recovery to temperature conditions suitable for striped bass would have been even more recent. For example, many marine organisms with similar Canadian ranges as the striped bass enlarged their distributions northward during the hypsithermal period of approximately 3000–6000 yr ago (Pielou 1991). Lewis (1957) stated Raney's hypothesis that, based on comparisons of meristic counts in striped bass, colonization of Canadian drainages occurred through migration from the Chesapeake Bay. Our data neither support nor refute this hypothesis. It is possible that their founders originated from other mid-Atlantic migratory populations, such as those in the Hudson and Delaware rivers, or from a Grand Banks refugium, as Schmidt (1986) hypothesized for another sympatric anadromous species, Atlantic tomcod (*Microgadus tomcod*).

It is possible that the high degree of mtDNA divergence observed between Canadian and southern striped bass populations reflects the nature of the mtDNA variation that was detected. Multiple variants in the length of the mtDNA molecule and a concomitant lack of base substitutions argue for a rapid generation of mtDNA size polymorphisms in striped bass. The molecular characterization of mtDNA length variants in striped bass, tandem duplications (Wirgin et al. 1989), the hypothesized molecular mechanisms whereby mtDNA length variants are generated in fish (Buroker et al. 1990), and the high frequency of heteroplasmy for mtDNA length variants in striped bass populations (Wirgin et al. 1989, 1990b) all argue for the frequent generation of variant mtDNA length genotypes in these populations. Generation of mtDNA length genotypes may have been a frequent and recent event(s) in some Canadian populations given their elevated frequency of heteroplasmy. If that is true, it is possible that the high frequency of the A length mtDNA genotype in Shubenacadie striped bass may have resulted from very recent single or multiple mutational event(s) in the Shubenacadie population. Yet the frequency of heteroplasmy was considerably lower in the Shubenacadie population than seen in striped bass from the Gulf of St. Lawrence. It is possible that the high frequency of larger mtDNA genotypes, A in the Shubenacadie and B in the Miramichi–Tabusintac, may have resulted from strong selective pressure for larger mtDNA genotypes in northern populations. However, an opposite trend was detected in American shad, in which the largest mtDNA genotypes and the highest frequency of heteroplasmy were detected in southern populations (Bentzen et al. 1988), observations which argue against a selective advantage of large mtDNA genotypes and an increased incidence of the generation of length variants in northern populations.

Assuming that individual mtDNA length genotypes arose multiple times within the species' range and perhaps within single populations, it could be argued that mtDNA length genotypes may not be informative in discerning the long-term matriarchal ancestry of striped bass populations. However, the requirements of genetic marks for fishery management are not as demanding. The fact that geographically proximal populations of striped bass exhibited significant differences in frequencies of mtDNA length genotypes, that these mtDNA length frequencies are temporally stable within a single population over several year classes (I. I. Wirgin and J. R. Waldman, unpublished data), and that hatchery-reared progeny of catalogued broodstock always exhibited the mtDNA length genotype of their maternal parent (Wirgin et al. 1989; Chapman 1990) argue for the utility of mtDNA length variation in the identification of striped bass stock structure.

Mark–recapture studies conducted over three decades have confirmed that U.S. and Canadian striped bass stocks do cooccur within the Bay of Fundy (Nichols and Miller 1967; Williamson 1974; Melvin 1978; Boreman 1986; Rulifson et al. 1987; Waldman et al. 1990), the degree of abundance of southern migrants within the Bay of Fundy reflecting their strength in U.S. coastal waters (Bigelow and Schroeder 1953). It is likely that the occurrence of striped bass of U.S. origin in the Bay of Fundy is due to its suitability as a feeding area, as the bio-

energetic costs of such a lengthy route may be balanced by the Bay's high productivity. In the only reported instance of striped bass migration between rivers in the Gulf of St. Lawrence and U.S. spawning and coastal sites, a single 3-yr-old fish tagged in the Kouchibouguac River in March 1983 was recaptured 33 d later in the Wye River, Maryland (Hogans and Melvin 1984).

The utilization of Bay of Fundy waters by striped bass of probable U.S. ancestry does not imply significant genetic admixture of these stocks. Adult striped bass have recently been reported from at least four major tributaries of the Bay of Fundy, including the Shubenacadie–Stewiacke system, the Annapolis River, the St. John River, and the Peticodiac River. In contrast, significant numbers of larval and juvenile striped bass were collected only from the Shubenacadie–Stewiacke despite repeated efforts in the Annapolis, St. John, and Peticodiac rivers. Thus, is is possible that among these Bay of Fundy rivers, only the Shubenacadie–Stewiacke system currently supports an endemic striped bass population. Although adult striped bass with U.S. tags have been reported from the Shubenacadie River system (Rulifson et al. 1987), the extremely high incidence of mtDNA length genotype A, the absence of genotype D, and the extremely low frequency of the most common U.S. genotype C indicate reproductive isolation of Shubenacadie fish. Thus, tagging and genetic evidence suggests that some Bay of Fundy rivers support endemic stocks and migratory U.S. fish whereas others may support only migratory adult U.S. fish.

A comparison of mtDNA length genotypes between individual striped bass eggs and adults collected from these rivers would permit an evaluation of the significance of spawning on adult stocks. Recent advances in DNA technology, such as development of the polymerase chain reaction (PCR), should permit testing of the impact of limited spawning success in the Annapolis and St. John rivers on their adult stocks. Provided that sampling of individual eggs was sufficiently stratified temporally and spatially within each river to adequately represent its population of eggs, a comparison of mtDNA length genotypes between eggs and adults would permit an evaluation of the impact of spawning on adult populations. PCR amplification of the mtDNA control region, the likely site of mtDNA length variation, in individual striped bass eggs is possible and would allow for these analyses. A comparison of mtDNA length genotype frequencies in adult striped bass from the St. John, Annapolis, and southern populations should prove informative as to the genetic relatedness of these Canadian fish to U.S. populations.

Currently, population sizes in some of these Canadian rivers are low (Melvin 1991) and augmentation of these populations by hatchery stocking is being considered (Harrell 1991). It is important that the principles of conservation genetics be considered in planning these introductions. These include the maintenance of endemic gene frequencies and genetic diversity within these populations (Ryman 1991). Our results argue against the transfer of striped bass among these three regions, the Gulf of St. Lawrence, the Bay of Fundy, and the United States, due to the potential alteration of gene frequencies within recipient populations. Additionally, the absence of mtDNA differences between striped bass from the Miramichi and Tabusintac rivers does not necessarily indicate a lack of reproductive isolation among fish in Gulf of St. Lawrence rivers. There is a need for characterization of mtDNA genotype frequencies in other stocks within the Gulf of St. Lawrence, and in addition the Bay of Fundy. Finally, the striped bass population in the St.Lawrence River is currently at, or near, extinction. If efforts to restore this stock are to proceed, they would best be accomplished using native St. Lawrence River broodstock. However, if native St. Lawrence broodstock is unobtainable, then it would be informative to survey mtDNA genotypes in archived St. Lawerence samples. This would provide the opportunity to select fish from extant striped bass stocks that most nearly approximate the genetic characteristics of the native St. Lawrence stock.

Although we demonstrated the distinctiveness of mtDNA length genotypes in some of these populations, the mitochondrial genome constitutes only a small percentage (<1%) of the total genetic information in the organism. Given the availability and demonstrated polymorphism of striped bass specific single-copy nuclear DNA probes (Wirgin and Maceda 1991) and repetitive nuclear DNA probes (Wirgin et al. 1991), an analysis of polymorphic nuclear DNA sequences in striped bass from these Canadian populations would provide a more comprehensive, quantitative, and perhaps sensitive evaluation of the genetic distinctiveness of these stocks.

Acknowledgments

We thank Kim Robichaud and Bill MacEachern for assistance in sample collection. This study was supported by NIH grant ES 00260 to I.I.W. and the Department of Fisheries and Oceans.

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Using Growth/Mortality Trade-offs to Explore a Crayfish Species Replacement in Stream Riffles and Pools

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Mather, M. E., and R. A. Stein. 1993. Using growth/mortality trade-offs to explore a crayfish species replacement in stream riffles and pools. Can. J. Fish. Aquat. Sci. 50: 88–96.

A crayfish species replacement is underway in Ohio streams. We explore how growth, mortality, habitat use, and size influence the replacement of *Orconectes sanborni* by *O. rusticus*. In field cages, we assessed growth of crayfish. Both species grew faster when confined to riffles and *O. rusticus* grew faster than *O. sanborni* in this fast-water habitat. In another experiment, we tethered crayfish in riffles, shallow pools (<40 cm), and deep pools (>40 cm) to assess mortality. After 8 d, more crayfish were eaten in deep pools than in riffles or shallow pools. Given this finding, we predicted that crayfish should preferentially occupy high-growth, low-mortality riffles. To test this hypothesis, we quantified crayfish habitat use in sympatric streams, with and without fish predators. Crayfish did not prefer riffles. Apparently, juvenile crayfish do not assess specific benefits and costs using only food and fish predators. Other costs associated with riffles (e.g. passive drift, terrestrial predators, summer desiccation, physiological cost of position maintenance, etc.) must reduce riffle use. In addition, *O. rusticus* grows larger than *O. sanborni*. Because size confers advantages in biotic interactions, this critical finding may be the key to the replacement.

La substitution d'une espèce d'écrevisse s'effectue actuellement dans des cours d'eau de l'Ohio. Nous examinons comment le croissance, la mortalité, l'utilisation de l'habitat et la taille influent sur la substitution d'*Orconectes sanborni* par *O. rusticus*. Nous avons étudié sur le terrain la croissance des écrevisses en cage. Les deux espèces se sont développées plus rapidement lorsqu'elles étaient confinées à des rapides, et le taux de croissance d'*O. rusticus* était plus élevé que celui d'*O. sanborni* dans ce type d'habitat en eau vive. Au cours d'une autre expérience, nous avons confiné des écrevisses dans des rapides, des étangs peu profonds (<40 cm) et des étangs profonds (>40 cm) afin d'en évaluer la mortalité. Après 8 jours, les prédateurs avaient consommé plus d'écrevisses dans les étangs profonds que dans les rapides ou les étangs peu profonds. D'après ces résultats, nous avons émis l'hypothèse selon laquelle les écrevisses fréquentent de préférence les rapides où elles ont un rythme de croissance élevé et un faible taux de mortalité. Pour vérifier cette hypothèse, nous avons quantifié l'utilisation de l'habitat par l'écrevisse dans des cours d'eau sympatriques, avec et sans poissons prédateurs. Nous n'avons pas observé de préférence pour les rapides chez les écrevisses. Il semble que les écrevisses juvéniles n'évaluent pas les avantages et les désavantages particuliers uniquement en fonction de la nourriture et des poissons prédateurs. D'autres facteurs négatifs liés aux rapides (par exemple, la dérive passive, les prédateurs terrestres, le dessèchement estival, le coût physiologique du maintien de la position, etc.) doivent diminuer l'utilisation des rapides. De plus, *O. rusticus* atteint une taille supérieure à celle d'*O. sanborni*. Étant donné que la taille représente un avantage dans les interactions biotiques, cette importante découverte peut constituer l'élément clé de la substitution.

Received February 5, 1992
Accepted July 8, 1992
(JB394)

Reçu le 5 février 1992
Accepté le 8 juillet 1992

A species replacement is currently underway in central Ohio streams. Naturally occurring in Indiana, Kentucky, and western Ohio, *Orconectes rusticus*, a large, aggressive crayfish, is becoming increasingly common outside its native range and has been linked to declines of native crayfishes in Ontario (Crocker and Barr 1968; Berrill 1978), Wisconsin (Capelli 1982; Lodge et al. 1986; Olsen et al. 1991), and Ohio (Turner 1926; Jezerinac 1982; Butler and Stein 1985). Herein, we explore habitat-specific growth and mortality of *O. rusticus* and *O. sanborni*. If differential use of riffles and pools results in differential growth or mortality for either species, then habitat selection could provide insight into the replacement.

Without predators, animals should select habitats that provide the greatest net energetic return (Sih 1982a; Werner et al. 1983b). Predators may alter prey habitat use by forcing prey into habitats where they are less vulnerable (Fraser and Cerri 1982; Cooper 1984; Power et al. 1985; Foster et al. 1988). Habitat choice, in fact, may result from an explicit trade-off between predation risk and growth or feeding rates (Sih 1980; Mittelbach 1981; Cerri and Fraser 1983; Werner and Gilliam 1984; Holomuzki 1986; Gilliam and Fraser 1987; Holbrook and Schmitt 1988; Werner and Hall 1988). Predators also can reduce habitat profitability by reducing prey activity (Stein and Magnuson 1976; Sih 1982b; Holomuzki and Short 1988), feeding rate (Milinski and Heller 1978; Dill and Fraser 1984; Power et al. 1985; Prejs 1987), and ultimately growth (Rasmussen 1987; Dixon and Baker 1988). Inter- and intraspecific competition also may modify habitat choice. For example, superior competitors (Bovbjerg 1970) and larger congeners (Butler and Stein 1985; Rabeni 1985) modify crayfish distribution. Thus, habitat use depends on food, predators, competitors, as well as other sources of mortality.

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TABLE 1. Possible advantages and disadvantages of riffle, shallow pool, and deep pool for juvenile crayfish in headwater streams. + indicates a potential positive effect for crayfish; − indicates a potential negative effect.

		Pool	
	Riffle	Shallow	Deep
Abiotic features			
Current	Fast (−)	Slow (+)	Slow (+)
Passive Drift	Yes (−)	No (+)	No (+)
Summer Drying	Yes (−)	Yes (−)	No (+)
Percent Cover	Often high (+)	Could be limited by sandy patches over entire pool (−)	
Biotic features			
Fish predators	No (+)	No (+)	Yes (−)
Terrestrial Predators	Yes (−)	Yes (−)	No (+)
Insect abundance	Can be high (+)	Low (−)	Low (−)
Adult crayfish	Present (−)	Present (−)	Present (−)

Differences in physical characteristics between pools and riffles affect stream animal distribution and abundance (Huryn and Wallace 1987a). Riffles and pools differ consistently in physical habitat structure and fish community composition (Schlosser 1982) (Table 1). Riffles are shallower, faster, and often have more aquatic insects than pools (Schlosser 1987; Schlosser and Ebel 1989; True 1990); furthermore, low-order, midwestern stream riffles rarely have fish predators. An abundance of high-quality food and absence of fish predators should attract juvenile crayfish to riffles. Conversely, terrestrial predation, desiccation in midsummer, aggression-induced passive drift, and position maintenance may be costs associated with high-velocity riffles. Thus, crayfish residing in stream riffles and pools may experience differences in growth and mortality rates. Crayfish habitat use should reflect these benefits and costs.

To assess if habitat use could contribute to the replacement of *O. sanborni* by *O. rusticus*, we pursued the following objectives. First, using field cages, we quantified growth of the two crayfishes in riffles and pools. Second, using tethering experiments, we measured crayfish mortality in riffles and pools. Third, based on these estimates of habitat-specific growth and mortality, we made predictions about how crayfish should be distributed across habitats. Finally, to test these predictions, we sampled habitat-specific crayfish density and size in the field.

Methods

Habitat-Specific Growth

In field growth experiments, three sites (one site = one riffle and one pool) in Raccoon Creek (Licking County, Ohio), a second-order stream of the Licking River drainage, were used (Table 2). To compare growth between riffles and pools, crayfish were enclosed in 0.74 m (long) × 0.30 m (wide) × 0.15 m (high) cages; water flowed through upstream and downstream ends which were covered by 5-mm^2 hardware cloth. Substrate, taken from the stream, was added to make the cage flush with the stream bottom. Rocks (N = 10) were measured inside and outside the cage to ensure that substrate inside cages mimicked natural conditions. Cages (six in each of two habitats at three sites = 36 cages total) were placed in the center of each riffle and pool. Initially, cages were open at the top for 2 wk to allow algae and invertebrates to colonize.

After 2 wk, two juvenile crayfish of each species (15–16 mm carapace length (CL), 1:1 male to female ratio) were added (N = 4 crayfish per cage) and cages were covered with hardware mesh lids (5 mm^2). This density (16 individuals·m^{-2}) was higher than that normally encountered ($\bar{X}$ = 8 individuals·m^{-2}) but the total biomass per unit area in each cage (21.1 g·m^{-2}) was within the natural range found in central Ohio streams (0.0–27.6 g·m^{-2}). Before the experiment, crayfish were collected from nearby allopatric sites, measured (nearest 0.5 mm), wiped quickly with a dry towel, weighed (nearest 0.001 g), marked with acrylic paint, and isolated in individually marked, flow-through 7 × 7 cm containers. Crayfish were fed lettuce ad libitum until experiments began. *Orconectes rusticus* and *O. sanborni* juveniles in each cage were exactly matched by length (nearest 0.1 mm) to avoid any initial size advantage. Screens on cage ends and lids were cleaned every other day with a wire brush. Of the 36 cages, 30 remained in the stream during June–July 1988 (mean temperature = 15°C). After 5 wk of growth, remaining crayfish were weighed and measured as described above.

Habitat-Specific Mortality

To quantify habitat-specific mortality, crayfish were tethered in riffles (7–10 cm deep), shallow pools (<40 cm), and deep pools (>40 cm) at the same three sites. As with the growth experiments, all pools at this site had fish predators. Pools were divided by depth to separate the effects of deepwater predators (fish) from those of shallow-water predators, (wading birds, raccoons). A fourth treatment, set up in a laboratory stream tank, assessed tether loss. Because time and crayfish availability prevented the use of both species, only the native crayfish *O. sanborni* was tethered. Other experiments (Mather 1990) support the assumption that same-sized *O. sanborni* and *O. rusticus* suffer similar predatory mortality when movement is restrained.

Juvenile crayfish (15–16 mm CL) were held in the laboratory and fed lettuce, liver, and fish ad libitum for about 2 wk. In the laboratory, a small snap swivel was superglued to the dorsal midpoint of the carapace to which 20 cm of transparent monofilament line was tied. In the field, during October and November, juvenile crayfish were attached, via this line, to stakes, 1 m apart, set up along several transects that bracketed the center of each habitat. The area 1 m from shore was avoided to eliminate desiccation from water-level fluctuations. In both pools and riffles, all tethered crayfish had access to shelter, i.e. either large substate ($\bar{X}$ = 12 cm, longest side) or small substrate ($\bar{X}$ = 3 cm, longest side). Because substrate size did not affect survival, large and small shelter treatments were combined (t-test on difference, $P > 0.05$). For each of three replicates, 10 crayfish were tethered in the riffle and 20 crayfish in the pool (10 large shelter + 10 small shelter).

To assess tether loss, crayfish, handled as above, were attached to a tile in riffles and pools of a laboratory stream tank (for a description, see Mather 1990). Tethered animals behaved normally (M. E. Mather, pers. obs.) as has been documented elsewhere (McIvor and Odum 1988; Hay et al. 1989). Each crayfish was checked at 2, 5, and 8 d. No stakes were lost; no

TABLE 2. Physical characteristics of sites used in caging experiments, tethering experiments, and field test. Manipulations tested growth (summer 1988) and mortality (fall 1989) in three riffles and three pools in Raccoon Creek (RAC). Field tests quantified habitat-specific crayfish size and density in South Fork of Licking River (SFL) and North Fork of Licking River (NFL). For density, two riffles and two pools were sampled in each river in both July and September 1988. For size, one riffle and one pool were sampled in each river in both July and September 1988. Predatory fishes included smallmouth bass (*Micropterus dolomieu*), rock bass (*Ambloplites rupestris*), and creek chub (*Semotilus atromaculatus*) ≥ 150 mm total length.

		Habitat			
	Mean predator density in pools	Riffle ($\bar{X}$)		Pool ($\bar{X}$)	
River	(no.·500 m^{-2})	Width (m)	Depth (m)	Width (m)	Depth (m)
RAC	4.5	14.0	0.1	20.0	0.73
NFL	0	7.7	0.1	8.6	0.45
SFL	5.8	5.1	0.1	9.1	0.30

tethers were broken. If the snap swivel had only a small piece of carapace attached, we assumed that the crayfish had been eaten by a predator. All tethered crayfish were held for 2 d in the laboratory before being placed in the stream. No molting was observed during this time or in trials of the stream tank treatment. Consequently, we believe that no crayfish escaped their tethers by molting during this field experiment. These tethering experiments were completed in October and November 1989 (at about 10°C).

Field Pattern

To assess habitat-specific field patterns, we first calculated a cost–benefit ratio for *O. sanborni* (Werner and Gilliam 1984). To calculate the cost–benefit ratio for *O. sanborni*, habitat-specific growth and mortality data from the above-described cage and tethering experiments were used. With this, we predicted crayfish habitat use. Next, using field sampling, crayfish density and size in each habitat were quantified. To document habitat-specific size and density, two second-order streams in the Licking River with sympatric crayfish populations were sampled. The South Fork of the Licking River (Licking County, Ohio) has a substantial fish predator population and the North Fork of the Licking River (Licking and Knox counties, Ohio) has no fish predators (Table 2).

Riffles and their adjacent pools were sampled by enclosing each with block nets; all of the riffles but only 17 m of the pool were sampled (resulting in similar riffle and pool sample areas). Enclosed areas were repeatedly kick-seined (N = 180 min effort per sample) and all crayfish collected, identified, and measured. In July and September 1988, at each site, one pool and one riffle were sampled for crayfish size (N = 1); at the same time, an additional riffle and pool (N = 2) were sampled for density. Crayfish density was analyzed using a three-way repeated measures analysis of variance (ANOVA) in which effects of fish, crayfish species, and habitat were tested. In this analysis, time was used as a repeated measure and the site populations were the experimental unit. Crayfish size was analyzed using a three-way ANOVA that tested the effects of fish, crayfish species, and habitat on log-transformed carapace lengths. Because crayfish size was measured at only one riffle and one pool, in the size analysis, the individual crayfish, not the site population, was the unit of measure. This analysis has limited generality but provides one opportunity to examine crayfish size distribution in the field. All effects discussed below were significant ($P < 0.05$).

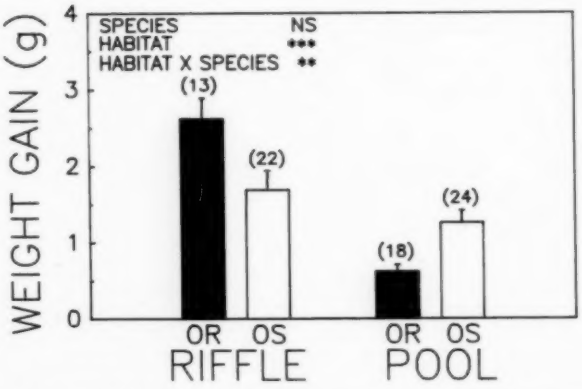

FIG. 1. Weight gain of juvenile (15 mm CL) *O. rusticus* (OR) and *O. sanborni* (OS) during 5 wk during June–July 1988 in riffles and pools of Raccoon Creek, Licking County, Ohio. Data are means ± 1 SE. Crayfish were recovered from 30 cages. Numbers of crayfish remaining at experiment's end are in parentheses; initially, 30 of each species were placed in each habitat. Because growth among the three locations within Raccoon Creek did not differ (ANOVA, $P > 0.05$), data from all sites were combined. See Table 3 for the results of two-way ANOVA used to assess how species and habitat influence growth. *$P \leq 0.05$; **$P \leq 0.001$; ***$P \leq 0.0001$. Increases in length and weight yielded similar conclusions, so only weight data were presented.

Results and Discussion

Growth

Results

From cage experiments, two important results emerged. First, riffle crayfish, regardless of species, grew more rapidly than pool crayfish (Fig. 1; Table 3). Second, the invader *O. rusticus* grew faster than the native *O. sanborni* in riffles and more slowly than *O. sanborni* in pools (Fig. 1; Table 3).

Discussion

Either food quantity or food quality may permit riffle-dwelling crayfish to grow faster than pool-dwelling ones. As opportunistic omnivores consuming mollusks (Covich et al. 1981; Crowl and Covich 1990; Alexander and Covich 1991), aquatic insects (Lorman 1975; Capelli 1980), macrophytes (Lorman 1975), algae (Capelli 1980), leaf litter (Huryn and Wallace

TABLE 3. Main effects and first-order interactions of ANOVA measuring how species and habitat influence weight gain of *O. rusticus* and *O. sanborni* during a 5-wk cage experiment in Raccoon Creek, Licking County, Ohio, during June–July 1988. $*P \leq 0.05$; $**P \leq 0.001$; $***P \leq 0.0001$. Data correspond to Fig. 1. Overall degrees of freedom are as follows: model df = 3; error df = 73; total corrected df = 76. Overall ANOVA SS = 32.19.

Effect	df	SS	*F*	*P*
Species	1	00.37	00.47	0.49
Habitat	1	26.74	33.40	0.0001***
Species × habitat	1	11.23	14.45	0.003**

1987b), and juvenile crayfish (Capelli 1980), crayfish diet varies with season, depth, size, and sex (Lorman 1975; Capelli 1980). However, crayfish are not indiscriminate foragers (Lorman 1975). Animal matter contains more calories per gram than plant material and often more stream insects occur in riffles than pools (Schlosser 1987; Schlosser and Ebel 1989; True 1990). At our sites, True (1990) found that riffle crayfish of both species consumed more macroinvertebrates than pool crayfish. Thus, when confined to cages, food quality was likely responsible for the high growth of riffle crayfish. Although cages may have reduced current velocity slightly, velocities inside cages were within the range in natural riffles. Hence, cages themselves were not the cause of these trends.

Although *O. rusticus* and *O. sanborni* did not differ in overall growth, *O. rusticus* grew faster than *O. sanborni* in riffles and more slowly than *O. sanborni* in pools. These growth differences could be caused by ecological, behavioral, or physiological mechanisms. First, when free-ranging, the invader *O. rusticus* actively selects more heptageniids, a large riffle invertebrate, than does *O. sanborni* (True 1990). Thus, the invader may be attaining a larger size in the riffle by differentially exploiting high-quality riffle food. Second, *O. rusticus* is more active than *O. sanborni* in a laboratory-simulated stream (Mather 1990); similarly, unrestrained *O. rusticus* feed at a higher rate than native crayfish (Butler and Stein 1985; True 1990). Both of these findings suggest that higher overall activity and more efficient foraging behavior may help *O. rusticus* exploit the riffle more efficiently and thereby grow faster. *Orconectes rusticus*, the innately more active species (Mather 1990), also may have a higher overall metabolic rate which allows this invader to take advantage of a good habitat but which extracts higher maintenance costs, thereby limiting growth, in less profitable habitats. That the native *O. sanborni* grows faster than *O. rusticus* in pools, the habitat where risk from fish predation is high, also could have important implications for the replacement.

Some mortality occurred in growth cages (see Fig. 1). This mortality, of unknown causes, occurred across all cages. Hence, density did not differ across treatments and did not contribute to growth differences. In summary, the growth difference between crayfish species likely occurs because *O. rusticus* (1) eats higher quality food, (2) feeds more actively, and (3) maintains a higher metabolism. In another system, differential feeding efficiency also contributed to a species replacement (McComas and Drenner 1982). In Ohio streams, *O. rusticus* exploited high-growth riffles better than *O. sanborni* and grew larger; thus differential growth likely contibutes to the replacement of *O. sanborni* by *O. rusticus*.

Mortality

Results

In the laboratory-simulated stream, no crayfish broke their tethers. More crayfish died in deep pools than in shallow pools or riffles (Fig. 2, ANOVA, SS = 4347.6, $F = 6.5$, $P = 0.03$). Survival in the two shallow-water habitats did not differ (Fig. 2) but more crayfish died in these habitats than in the laboratory stream tank.

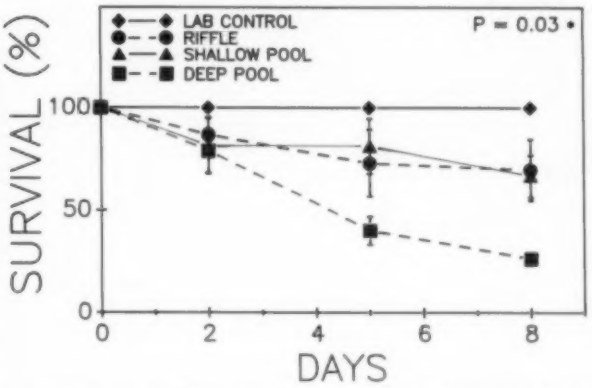

FIG. 2. Survival of juvenile *O. sanborni* (N = 10–20 crayfish per habitat for each of three replicates) tethered in riffles (7–14 cm), shallow pools (<40 cm), deep pools (>40 cm), and a laboratory stream tank during 8 d in fall 1989. Data are means ± 1 SE. Because survival in the three sites within Raccoon Creek did not differ (ANOVA, $P > 0.05$), data from all sites were combined. The results of a repeated measures ANOVA using time as the repeated measure and assessing how habitat influenced survival are indicated.

Discussion

Predators (fish, avian, and mammalian) may influence habitat use. For example, without fish predators, juvenile stream fishes of all taxa reviewed by Schlosser (1987) prefer stream pools, but with predators they occur mostly in riffles. Similarly, armored catfish avoid the food-rich shallow areas of a Panamanian stream, probably because bird predators occur inshore (Power 1984). In our study, deepwater mortality was caused by fish predators; terrestrial predators, i.e. wading birds and racoons, most likely caused the mortality in shallow pools and riffles. Shelters may modify risks, but all tethered crayfish had access to shelter and, regardless, many were eaten. Hence, for crayfish, predation risk may be an ever-present danger. At out study site, deep pools, where fish predators live, were more dangerous for juvenile crayfish than shallow-water habitats, where avian and mammalian predators forage. Conversely, at other sites with large populations of terrestrial predators and no fish predators, riffles and shallow pools could be the most dangerous habitat. Clearly, the relative importance of each type of predation varies spatially and temporally with changes in the vertebrate community.

Because only *O. sanborni* was tethered, habitat-specific mortality can be calculated only for this native crayfish. However, we can speculate about mortality of *O. rusticus*. Only

TABLE 4. Ratio of mortality (proportion dead through 8 d; see Fig. 2) to growth (weight gain in g·8 d^{-1}; see Fig. 1) for riffles and pools in Raccoon Creek, a second-order Ohio stream with predatory fishes. Mortality was assessed from field experiments (fall 1989) for *O. sanborni*. Growth was measured in cage experiments for both species in summer 1988. OR = *O. rusticus*; OS = *O. sanborni*. (Note: theory predicts that animals should minimize the mortality/growth ratio.)

	Riffle		Pool	
	OR	OS	OR	OS
Mortality	—	0.25	—	0.75
Growth	0.52	0.35	0.12	0.25
Mortality/growth	—	0.71	—	3.00

three outcomes are possible: *O. rusticus* could suffer (1) higher, (2) equal, or (3) lower mortality than the native. No evidence (reviewed below) exists that *O. rusticus* is more susceptible to predators than native orconectid crayfish; hence, this first possible outcome can be dismissed. Same-sized *O. rusticus* and *O. sanborni* are equally vulnerable to smallmouth bass (*Micropterus dolomieu*, previously *Micropterus dolomieui*) predation in one study in a simulated stream tank (Mather 1990). However, in another study using aquaria, large, young-of-year *O. rusticus* (14 mm CL) survived bass predation better than small *O. sanborni* (12.5 mm CL; Butler and Stein 1985). Because larger (3.5 mm) young-of-year *O. rusticus* are eaten less often than smaller *O. sanborni* (Mather 1990), these last results may also reflect a size rather than a species effect. However, from these existing data, whether *O. rusticus* is equally or less vulnerable than *O. sanborni* is unclear. Thus, in our discussion, we will assume that the invader *O. rusticus* suffers either similar or lower predatory mortality than *O. sanborni*.

Field Pattern

Predictions

Using our assessment of costs and benefits based on fish predators and food, the ratio of mortality to growth was minimized in riffles (Table 4). Thus, based on food and fish predation, for the native *O. sanborni*, the riffle should be a four-fold more profitable habitat than the pool.

Discussion

Others have found that foragers trade off energetic return and predation risk when these two factors conflict (Sih 1980; Werner et al. 1983a; Werner and Hall 1988). How foragers assess these trade-offs is not clear. They may evaluate food and danger independently (Cerri and Fraser 1983), simultaneously (Werner and Gilliam 1984; Gilliam and Fraser 1987), or using a repertoire of flexible responses (Holbrook and Schmitt 1988). Based on growth and mortality as measured here, crayfish should preferentially occupy riffles.

Our prediction that the riffle is the most energetically profitable habitat, we believe, accurately reflects habitat-specific costs and benefits to juvenile crayfish based on food and fish predators. For many organisms, habitat use can be accurately predicted by examining only these two factors (Cerri and Fraser 1983; Werner et al. 1983a; Gilliam and Fraser 1987; Holbrook and Schmitt 1988). Hence, the assumption that food and fish predators are of critical importance is a reasonable one. The field data that we used for our predictions are strong. Thus, agreement between field data and our predictions will confirm the overwhelming importance of these factors; disagreement between field data and our predictions, in turn, will demonstrate that other growth and mortality factors play a central role in juvenile crayfish habitat choice.

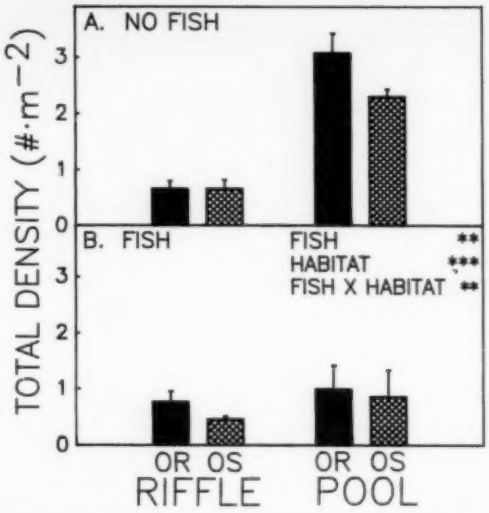

FIG. 3. Density of *O. rusticus* (OR) and *O. sanborni* (OS) in riffles and pools collected during a field test of sites (A) without fish predators (North Fork of the Licking River, Licking and Knox counties, Ohio) and (B) with fish predators (South Fork of Licking River, Licking County, Ohio) during July–September 1988. Data are means ± 1 SE. Two replicates of each habitat at each site were sampled at two times. The results of a repeated measures ANOVA that tested the effect of habitat, species, and fish treatment (see Table 5) are indicated; $*P \leq 0.05$; $**P \leq 0.001$; $***P \leq 0.0001$.

Field Pattern: Density

Results

Crayfish did not shift into the riffle when fish were present as we predicted (Fig. 3). Rather, crayfish densities were greatest in pools for both species with and without fish (Fig. 3; Table 5). Overall densities were lower with fish primarily because pool densities with fish were lower than pool densities without fish (Fig. 3; Table 5).

Discussion

Based on cage/tethering experiments, juvenile crayfish should select the high-growth, low-predation-risk riffles. Consequently, higher crayfish densities should occur in riffles especially with fish predators in pools. However, both with and without fish, for both species, pool densities exceeded riffle densities. Why did our cage/tethering experiments provide so little insight into crayfish habitat choice? First, position maintenance in riffles may require substantial energy. For juvenile fish, for example, costs of foraging in riffles outweigh benefits (Schlosser 1987). Second, fast current in riffles may increase the probability of passive drift. Although *O. rusticus* is more streamlined than *O. propinquus* (Maude and Williams 1983), water velocity may still constrain high occupancy of the riffle. When a crayfish loses its footing in the riffle, often as a result of inter- or intraspecific aggression, it passively drifts. As a result, the probability of consumption by fish in downstream pools is substantially increased. In our simulated stream tank, predation following passive drift was frequently observed (Mather 1990). Third, terrestrial predators may deter crayfish from inhabiting riffles. In summary, we believe that juvenile

TABLE 5. Main effects and first-order interactions of ANOVA testing how fish, species, habitat, and time affect total crayfish density in the South and North forks of the Licking River (Licking and Knox counties, Ohio) during June–September 1988. Higher order interactions were not significant. $*P \leq 0.05$; $**P \leq 0.001$; $***P \leq 0.0001$. Data correspond to Fig. 3. Overall degrees of freedom are as follows: model df = 14; error df = 17; corrected total df = 31. Overall ANOVA SS = 32.15.

Effect	df	SS	*F*	*P*
Fish	1	6.69	19.75	0.0004**
Species	1	0.76	2.25	0.15
Habitat	1	11.00	32.49	0.0001***
Time	1	0.24	0.72	0.41
Fish × species	1	0.06	0.16	0.70
Fish × habitat	1	5.88	17.36	0.0006**
Fish × time	1	0.17	0.52	0.48
Species × habitat	1	0.18	0.53	0.48
Species × time	1	0.17	0.52	0.48
Habitat × time	1	0.28	0.84	0.37

crayfish are not assessing habitat-specific benefits and costs using only food and fish predators. Other costs encountered in the riffle must outweigh advantages associated with high food quality and refuges.

Field Pattern: Size

Results

The invader *O. rusticus* was larger than the native *O. sanborni* within each site (Fig. 4A; Table 6), within each habitat (Fig. 4B; Table 6), and especially in riffles at the site with fish predators (Fig. 4B). At the site with fish predators, crayfish size was reduced overall (Fig. 4A; Table 6) and for both species (Fig. 4B; Table 6). At the site with fish predators, riffle crayfish were larger than pool crayfish (Fig. 4B; Table 6); without fish predators, this trend was reversed and riffle crayfish were smaller than pool crayfish (Fig. 4B; Table 6).

Discussion

Unlike our cage results, species sizes consistently differed in the stream; in all comparisons, *O. rusticus* was larger than *O. sanborni*. As in cages, this difference was most pronounced in riffles and at the site with fish. An overall difference would appear in the field samples, but not necessarily within the confines of field cages, if *O. rusticus* is more active and forages over wider areas (Mather 1990). Hence, *O. rusticus* may attain its larger size, in part, via increased foraging efficiency in both riffles and pools.

Our field data demonstrate that variation exists in crayfish size both with predation intensity and between habitats. At the site with fish predators, crayfish were smaller. Because size was measured at only one site with fish predators and one site without fish predators, we cannot generalize about the impact of fish predators on crayfish size and growth. However, other studies suggest that fish predators may negatively affect crayfish size. For example, fish predators (1) reduce crayfish activity and consequently feeding efficiency (Stein and Magnuson 1976; Mather 1990), (2) force crayfish into less profitable microhabitats (Stein 1977; True 1990), (3) may prevent crayfish from being active foragers on invertebrates (True 1990), and/or (4) selectively prey on certain sizes of crayfish (Stein 1977). All of these fish-induced effects will reduce crayfish size. Whether this trend applies to all sites with fish predators requires further examination.

How do the results from the field sampling compare with the crayfish size pattern in the growth experiments? As predicted by our experiments, at the site with fish, larger crayfish occupied riffles, but at the site without fish, smaller crayfish occupied riffles. Free-ranging pool crayfish likely consume more food than pool crayfish constrained by either fish predators or cages. Growth is only one measure of habitat quality and other factors also may be important; but by assessing habitat on this factor alone, two trends emerge: (1) foraging efficiency of pool

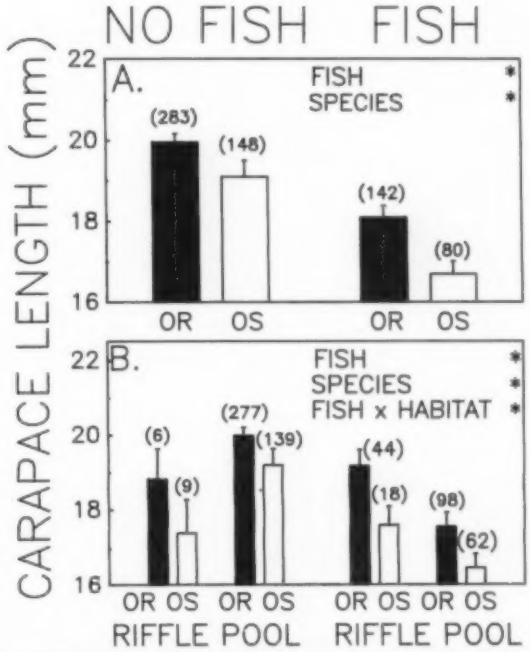

FIG. 4. Carapace length of *O. rusticus* and *O. sanborni* in riffles and pools without fish predators (North Fork of the Licking River, Licking and Knox counties) and with fish predators (South Fork of Licking River, Licking County, Ohio) during July–September 1988. (A) Effects of fish predators on carapace length of *O. rusticus* and *O. sanborni* (pools and riffles combined); (B) effect of fish on carapace length of *O. rusticus* and *O. sanborni* in riffles and pools. For both panels, bars on the left half show sites without fish and bars on the right half show sites with fish. Data are means ± 1 SE. The experimental unit is the individual crayfish. Sample size is indicated in parentheses. Results of a three-way ANOVA are presented (Table 6); $*P \leq 0.05$; $**P \leq 0.001$; $***P \leq 0.0001$. Note that the y-axis does not begin at zero.

TABLE 6. Main effects and first-order interactions of ANOVA testing how fish, species, and habitat influence crayfish size (carapace length) in the South and North forks of the Licking River (Licking and Knox counties, Ohio), during June–September 1988. Higher order interactions were not significant. *$P \leq 0.05$. Data correspond to Fig. 4. Overall degrees of freedom are as follows: model df = 7; error df = 645; corrected total df = 652. Overall ANOVA SS = 929.18.

Effect	df	SS	*F*	*P*
Fish	1	54.58	3.69	0.05*
Species	1	59.76	4.04	0.04*
Habitat	1	0.06	0.00	0.95
Fish × habitat	1	81.56	5.51	0.02*
Fish × species	1	0.64	0.04	0.84
Species × habitat	1	2.92	0.20	0.66

crayfish may be reduced with fish and (2) both food quantity and food quality are important to free-ranging crayfish.

Conclusions

Role of Habitat

In habitats with fish, these predators probably affect crayfish in two ways. First, fish may reduce crayfish survival by consumption. Second, fish may reduce crayfish growth by altering crayfish activity and foraging efficiency. Crayfish growth was higher and predatory mortality was lower in riffles with fish predators. But apparently, costs unrelated to fish predators may negate advantages associated with this habitat because we found more crayfish in pools than in riffles throughout the field samples (two pools + two riffles per stream × two streams = eight riffle pool complexes, each sampled twice). Specifically, terrestrial predators may be an important source of mortality for crayfish in some streams. Not only do birds and mammals focus on a different habitat (shallow pools and riffles vs deep pools), but unlike gape-limited fish predators, they likely select the largest adult crayfish. Thus, when terrestrial predators are abundant, crayfish do not enjoy a size refuge and terrestrial predators will have different effects on habitat-specific crayfish densities and sizes than fish predators. Dangers associated with high current (passive drift, physiological costs, drying during the summer and resultant density-dependent interactions, cannibalism, etc) also may be important in habitat choice of juvenile crayfish. The high-growth, low-mortality riffle may be a good place to be only if (1) terrestrial predators are limited, (2) position can be maintained, and (3) aggression can be repelled. Riffles may act simply as nursery areas in which juveniles grow through their window of vulnerability to fish predators.

Role of Species

The replacement of native crayfish by *O. rusticus* has been widely studied. The first studies documented the unambiguous initial pattern, i.e. *O. rusticus* was introduced, became established immediately, and rapidly increased in number whereas native crayfish abundances declined concurrently (Turner 1926; Rhoades 1944, 1962; Crocker and Barr 1968; Berrill 1978; Capelli 1982; Jezerinac 1982). In the next group of studies, mechanisms for this seemingly simple field pattern were sought. The roles of abiotic factors (Capelli and Magnuson 1983; Flynn and Hobbs 1984), reproductive interference (Capelli and Capelli 1980; Tierney and Dunham 1984; Butler and Stein 1985), and competition (Capelli and Munjal 1982; Flynn and Hobbs 1984; Butler and Stein 1985) were examined. Although each of these factors provides partial information about mechanisms, exactly how *O. rusticus* replaces its congeners is still unclear.

The role of interspecific competition in the replacement, especially, has received wide attention. Species-specific differences in competitive ability exist among orconectid crayfish (Bovbjerg 1970; Capelli and Munjal 1982). In the field, *O. rusticus* has increased at the expense of native crayfishes in just 5 yr in several Wisconsin lakes (Capelli 1982). But in Ohio, *O. sanborni*, a native more aggressive than other native crayfishes, can hold its own with *O. rusticus* when equal sized. This even match between Ohio crayfish species may explain why, in central Ohio streams, the replacement has not led to the rapid extirpation of *O. sanborni* in sympatric systems.

The third wave of studies (Butler and Stein 1985; Lodge et al. 1986) showed that long-term interactions between *O. rusticus* and native crayfish were complex and subtle. This *O. rusticus* – native crayfish replacement, initially classified as straightforward, now appears to have multiple causes and a variety of outcomes depending on the native crayfish species and system-specific characteristics (Mather 1990; Olsen et al. 1991).

Role of Size

Why is *O. rusticus* consistently larger than *O. sanborni*? *Orconectes rusticus* is a more efficient forager in both riffle and pool. In addition, *O. rusticus* juveniles leave the female 2 wk before other orconectid crayfishes, although female *O. rusticus* oviposits simultaneously with other orconectid crayfish (Corey 1988). In other systems, size-structured interactions influence physiological responses, feeding rate, competitive ability, and susceptibility to predation in phytoplankton, zooplankton, and fish (Stein et al. 1988). For crayfish, size confers an advantage in survival (Stein 1977) and reproduction (Stein 1976). Juveniles and adults often segregate by habitat. These life stages may segregate in response to differences in feeding efficiency and vulnerability to predators (Brandt 1980; Mittelbach 1981; Schlosser 1982; Baltz and Moyle 1984; Sandheinrich and Hubert 1984; Moyle and Vondracek 1985). In addition, cannibalistic adults may force juveniles into energetically less profitable habitats (Fraser and Cerri 1982; Rabeni 1985; Kneib 1987; Foster et al. 1988). Large crayfish are extremely aggressive and cannibalistic toward small crayfish (Mather 1990). In fact, in our system, large crayfish (>25 mm CL) increase activity, habitat changes, fights, and swimming escapes of small crayfish (15 mm CL; Mather 1990). In our field samples, invulnerable crayfish (>25 mm CL) were distributed relatively evenly throughout all habitats (Mather 1990). Hence, in our field study, the adult threat was ubiquitous; small crayfish could not escape large ones by simply shifting habitats (Mather 1990). *Orconectes sanborni*, more susceptible to large-crayfish aggression both because of smaller size and inherent species characteristics (Mather 1990), should suffer most from aggression, regardless of habitat.

Potential Mechanisms for the Replacement

Orconectus rusticus appears to grow faster in the riffle and be larger across both habitats. Because increased size also reduces vulnerability to predation (Mather 1990), all factors that lead to larger size of *O. rusticus* including habitat use, foraging behavior (greater food consumption and more heptageniids in the diet), and life history (earlier escape from the female) are important. Thus, habitat use, via size-structured interactions, contributes to the replacement of *O. sanborni* by *O. rusticus*.

Acknowledgements

Many people contributed to the completion of this research. Bob Essman and Robert Grandstaff allowed us to work in their streams. A National Science Foundation Dissertation Improvement Grant, Sigma Xi, and the Ohio Cooperative Fish and Wildlife Research Unit provided support for this research. Dave Armstrong, Tony Minamyer, Paul Minillo, and Paula Edersheim provided technical assistance. Jay Callender loaned us his house during the growth experiments. We thank Jim Mather and all members of the Aquatic Ecology Laboratory for their help. Comments by Andy Turner, Billie Kerans, Nick Collins, Alan Covich, and two anonymous reviewers improved the manuscript.

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Accumulation of PCBs by Lake Trout (*Salvelinus namaycush*): An Individual-Based Model Approach[1]

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Madenjian, C. P., S. R. Carpenter, G. W. Eck, and M. A. Miller. 1993. Accumulation of PCBs by lake trout (*Salvelinus namaycush*): an individual-based model approach. Can. J. Fish. Aquat. Sci. 50: 97–109.

To explain the variation in growth and in concentration of polychlorinated biphenyls (PCBs) among individual fish, an individual-based model (IBM) was applied to the lake trout (*Salvelinus namaycush*) population in Lake Michigan. The IBM accurately represented the variation in growth exhibited by the different age classes of lake trout. Uncertainty analysis of the IBM revealed that mean PCB concentration for the lake trout population was most sensitive to PCB concentration in their prey. The variability in PCB concentration among lake trout individuals was not adequately explained by the IBM, unless variation in prey fish PCBs was included in the model. To accomplish this, the simulated lake trout population was divided into subsets subjected to different levels of PCB concentration in the prey fish. Thus, model results indicated that variability in prey fish PCB concentration was an important component of the variation in PCB concentration observed among individual lake trout comprising the Lake Michigan population.

Les auteurs ont utilisé un modèle de type individuel afin d'expliquer les écarts de la croissance et de la concentration des biphényles polychlorés (BPC) entre les individus d'une population de touladi (*Salvelinus namaycush*) du lac Michigan. Le modèle a permis de représenter adéquatement les écarts de croissance notés entre les diverses classes d'âge des touladis. Une analyse d'incertitude du modèle a montré que la concentration de BPC des proies était le facteur influant le plus sur la concentration de BPC de la population de touladi. La variabilité de cette concentration entre les touladis n'était pas expliquée de façon adéquate par le modèle à moins que l'on ne tienne compte aussi de la variation de cette concentration chez les proies. À cette fin, la population simulée de touladi a été divisée en sous-ensembles en fonction des diverses teneurs en BPC des proies. Le modèle indiquait donc que la variabilité de la concentration des BPC des proies était un important facteur de la variation de la concentration des BPC notée chez les individus de la population de touladi du lac Michigan.

Received August 20, 1991
Accepted July 7, 1992
(JB191)

Reçu le 20 août 1991
Accepté le 7 juillet 1992

Accumulation of polychlorinated biphenyls (PCBs) by lake trout (*Salvelinus namaycush*) in Lake Michigan may hinder successful natural reproduction by these fish (Mac 1988) and may pose a health threat to people consuming lake trout (Cordle et al. 1982; Fein et al. 1984). Because of these concerns, PCB concentrations in lake trout have been monitored by state and federal agencies since the 1970's. The Michigan Department of Natural Resources (1991) and Wisconsin Department of Natural Resources (Masnado 1987) have issued consumption advisories for Lake Michigan salmonids. Furthermore, the U.S. Food and Drug Administration has imposed action levels for PCB concentration in fish to serve as guidelines for management of this problem (DeVault et al. 1985).

PCB accumulation in the "average" lake trout individual from Lake Michigan has been modeled by Weininger (1978), Jensen et al. (1982), and Thomann and Connolly (1984); however, the variation in PCB concentrations within the Lake Michigan lake trout population has yet to be modeled. Lake trout PCB concentration varies widely among individuals of the Lake Michigan population (Weininger 1978; Masnado 1987). During 1985, the Wisconsin Department of Natural Resources sampled PCB concentration in skins-on fillets from 125 individual lake trout (Masnado 1987). For a given length, lake trout PCB concentration showed considerable variation (Fig. 1). For example, for lake trout approximately 620 mm in total length, PCB concentration ranged from 1.5 to 6.0 mg/kg. Some of this variation was undoubtedly due to age differences among fish at this length.

Differences in diet within a cohort (fish of the same age) may also contribute to the variation in PCB concentration exhibited by the lake trout. The largest lake trout in a cohort may be more

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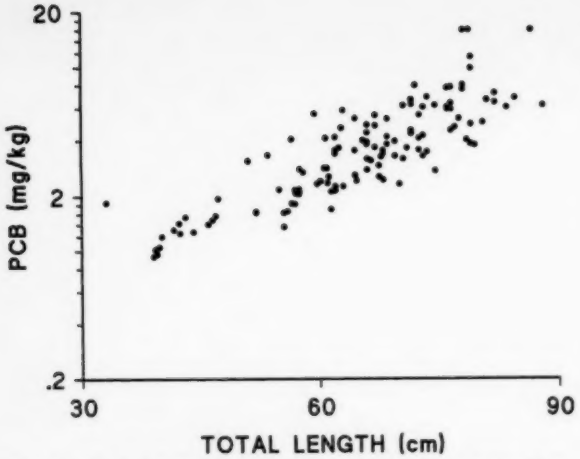

FIG. 1. Whole-fish PCB concentration versus fish total length for angler-caught lake trout from Wisconsin waters of Lake Michigan during 1985 (M. Miller, unpubl. data). Whole-fish PCB concentrations were estimated by applying a lipid correction of skins-on fillet PCB concentrations (see Lake Michigan Data section).

than 200 mm longer than the smallest lake trout within the cohort; larger trout may feed on substantially larger prey fish than smaller trout. PCB concentrations in prey fish vary between species, and PCB concentrations in prey fish generally increase with age (Weininger 1978).

The individual-based model (IBM) approach appears to be especially appropriate for addressing variation in PCB concentrations within a lake trout cohort. By applying the model to cohorts of various ages, the IBM can also be used to address variation in lake trout PCB concentrations due to age. Individual-based modeling is a relatively new computer simulation technique in which individual members of the population are tracked over time (Huston et al. 1988). Such an approach allows for interactions between individuals. IBMs have proven useful when predator–prey interaction at the level of individual fish is necessary to account for size variability shown within the piscivorous fish population. For example, first-year growth of several species of piscivorous fish depends on vulnerability of the prey fish (Adams and DeAngelis 1987). If the piscivore individual is sufficiently large relative to the prey fish, then that individual will grow relatively quickly on a fish diet. If prey fish are not vulnerable to an individual piscivore, then the piscivore will subsist on an invertebrate diet and grow at a relatively slow rate. Adams and DeAngelis (1987) applied an IBM to predict length–frequency distributions of young-of-the-year (YOY) largemouth bass (*Micropterus salmoides*).

In the present study, we will first determine if the IBM approach can explain the variation in size shown within the various age cohorts of lake trout in Lake Michigan. Next, we will determine if the IBM can explain the variation in Lake Michigan lake trout PCB concentrations when both age and size differences between lake trout are included in the model. If these two sources of variation are insufficient to explain all of the observed variation in the PCB concentrations, the following additional sources of variation will be entertained: (1) variation due to model parameter variability, (2) stochastic variation in PCB concentrations of prey, and (3) variability arising from bias in the degree of exposure to PCBs in the diet. The third source of variation can be paraphrased as follows: some lake trout are exposed to prey with relatively high PCB concentrations, while other lake trout in the population are exposed to prey with relatively low PCB concentrations.

Lake Michigan Background

The Lake Michigan population of lake trout has undergone dramatic changes in the last 50 yr. The population supported a stable commercial fishery from the 1880's through the early 1940's (Smith 1968). During the late 1940's, the population suffered a precipitous decline, which has been attributed to overexploitation by the commercial fishery and predation by the sea lamprey (*Petromyzon marinus*). The population was virtually extinct by the early 1950's. A lake trout stocking program was initiated in 1965 (Rybicki 1983) and has continued to the present time. Although lake trout stocked into Lake Michigan have failed to reproduce naturally, the stocking program has restored the population to a level that supports a valuable sport fishery.

PCBs were first detected in lake trout from Lake Michigan in the late 1960's (Masnado 1987). An ongoing surveillance program has been conducted since 1972. Based on composite samples of fish (averaging between 620 and 640 mm in total length), PCB concentration peaked in 1974 at 22.9 mg/kg and then declined exponentially to a value of 5.6 mg/kg in 1982 (DeVault et al. 1985). The current U.S. Food and Drug Administration action level for PCB in Great Lakes fish has been set at 2.0 mg/kg (DeVault et al. 1985).

Model

Our IBM for lake trout growth and accumulation of PCBs is a computer simulation model in which a set of individuals representing the population is tracked over several years of life in Lake Michigan (Fig. 2). The model was composed of three submodels: (1) a lake trout bioenergetics submodel, (2) a predator–prey encounter submodel, and (3) a PCB accumulation submodel. The bioenergetics submodel that we employed was identical to the bioenergetics model presented by Stewart et al. (1983) for lake trout in Lake Michigan, except for one modification. Whereas Stewart et al. (1983) assumed that lake trout consumed a fixed proportion (P) of their maximum consumption limit, we allowed individual lake trout to encounter individual prey fish and then consume their prey, provided certain conditions were met. This predator–prey encounter submodel was in effect from time of stocking to the time of first spawning at age 5. For the remainder of the simulated lake trout's life, each lake trout consumed a fixed proportion of the maximum consumption limit. The basis for the Stewart model is that growth (G) is equal to consumption (CONS) minus the sum of respiration (RESP), egestion (EG), excretion (EX), and specific dynamic action (SDA):

$$(1)\quad G = \mathrm{CONS} - (\mathrm{RESP} + \mathrm{EG} + \mathrm{EX} + \mathrm{SDA}).$$

All terms in equation 1 can be expressed as weight. See Stewart et al. (1983) for more details on each of the components of the bioenergetics submodel.

We used a predator–prey encounter submodel similar in form to that used by Madenjian (1991) for YOY walleye (*Stizostedion vitreum*, formerly *Stizostedion vitreum vitreum*) in western Lake Erie, except our submodel was considerably more complicated in regard to categories of prey fish, seasonality of prey fish availability, and changes in prey fish availability over the life history of the predator. During each simulated day, a lake trout

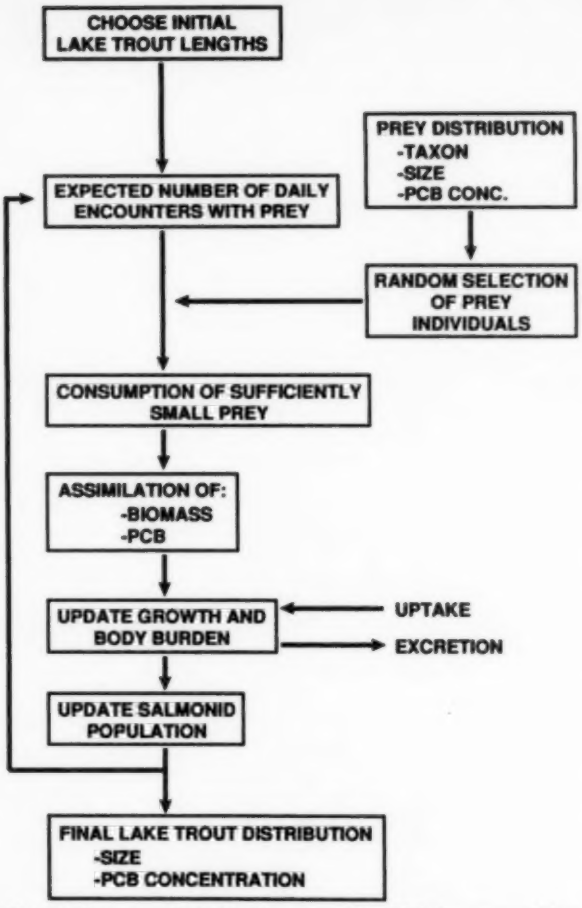

FIG. 2. Diagram of individual-based model (IBM) for accumulation of PCBs by lake trout.

encountered prey fish at a rate determined by a Poisson-distributed random variable. The mean of the Poisson distribution for encounter rate varied with age of lake trout and season (see Appendix 1). The number of encounters for a particular fish on a particular day was determined by randomly drawing from a Poisson distribution (see Madenjian 1991). For each encounter, the length of each prey fish was assigned by randomly selecting from a normal distribution with mean and standard deviation estimated from observations on prey fish populations in Lake Michigan (see Lake Michigan Data section). If the prey fish was small enough to be ingested, and if the consumption of the prey fish did not result in exceeding the maximum daily limit on consumption, then the prey fish was ingested (Fig. 2). Fish lengths reported in this paper are expressed as total lengths. Maximum daily consumption was modeled as a function of lake trout weight and water temperature (Stewart et al. 1983).

If the individual lake trout was unable to consume any prey fish on a particular day, then that individual received an invertebrate ration equivalent in mass to a fraction α of the maximum daily consumption limit. If the total mass of fish ingested by a lake trout individual was less than the fraction α of maximum daily consumption, then the lake trout received an invertebrate ration equal in mass to the difference between the fraction α of maximum daily consumption and the mass of fish ingested. α was set equal to 0.85 for the first 136 simulation days and then was assigned the value of 0.30 for the remainder of the simulation. Rough estimates for the values of α were provided by Stewart et al. (1983). These estimates were adjusted so that predicted and observed length–frequency distributions of lake trout were well matched.

Prey fish categories included in the model were YOY rainbow smelt (*Osmerus mordax*), yearling smelt, adult smelt, sculpins (slimy sculpin, *Cottus cognatus*, and deepwater sculpin, *Myoxocephalus thompsoni*, were sufficiently similar to be lumped in the same category), YOY alewife (*Alosa pseudoharengus*), yearling alewife, adult alewife, and YOY bloater (*Coregonus hoyi*). Based on food habit studies for Lake Michigan lake trout (Jude et al. 1987; D. Jude, Great Lakes Research Division, University of Michigan, Ann Arbor, MI, unpubl. data; G. Eck, unpubl. data), the maximum ratio, γ, of prey fish length to lake trout length at which the prey fish was still vulnerable to predation by the lake trout was assigned the value 0.4 for all prey fish categories. Weight–length relationships were needed in our IBM to convert from length to weight and vice versa (Adams and DeAngelis 1987; Madenjian and Carpenter 1991). Previous studies furnished the weight–length regressions for lake trout (R. Rybicki, Michigan Department of Natural Resources, Charlevoix, MI, unpubl. data), smelt (Bailey 1964), sculpin category (Rottiers 1965; Selgeby 1988), alewife (Stewart and Binkowski 1986), and bloater (Dryer and Beil 1968).

The PCB accumulation submodel was based on the model presented by Thomann (1989). Daily increment in a fish's body burden (ΔBB, micrograms PCB) was described as a balance between direct uptake from water (du, micrograms of PCB), uptake from food (ingest, micrograms PCB), and loss by excretion (excrt, micrograms PCB):

$$\Delta BB = du + ingest - excrt. \quad (2)$$

Direct uptake during one day was modeled as

$$du = k E_d C_w W^{0.8} \quad (3)$$

where E_d = direct uptake efficiency (dimensionless), C_w = PCB concentration in lake water (micrograms per litre), W = weight of lake trout (grams), and k is a constant which Thomann (1989) has calculated to be equal to 1.0 $L/g^{0.8}$.

Daily uptake from food was described simply as

$$ingest = E_f T_f \quad (4)$$

where E_f = assimilation efficiency of ingested PCB (dimensionless) and T_f = total amount of PCB ingested during the day (micrograms PCB). T_f was calculated by summing, for all of the prey items for an individual lake trout over the daily period, the products of PCB concentration (micrograms per gram) in the prey item and weight (grams) of the prey item. PCB concentrations in the prey items were based on information from Weininger (1978) and the Wisconsin Department of Natural Resources (M. Miller, unpubl. data) (see Appendix 2).

Daily excretion was modeled as

$$excrt = E_x k_x W^{-0.2} BB (\rho z)^{-1} \quad (5)$$

where E_x = excretion efficiency of assimilated PCB (dimensionless), W = lake trout weight (grams), BB = body burden (micrograms PCB), ρ = portion of whole-body weight composed of lipid (dimensionless), z = a dimensionless constant equal in value to the octanol–water coefficient (K_{ow}) for total PCB, and k_x is a constant which Thomann (1989) has calculated to be equal to 1.0 $g^{0.2}$. ρ was set equal to 0.155 (Rottiers and Tucker 1982). We assigned z the value of 10^6 because 6 is in

the middle of the range of base 10 logarithms of octanol–water coefficients for the most common PCB congeners (Burkhard 1984).

Thomann (1989) described the efficiency parameters (E_d, E_f, and E_x) as functions of contaminant K_{ow}. Jensen et al. (1982) and Thomann and Connolly (1984) used a value of 0.80 for E_f in their models. This value corresponded to a $\log_{10}$ (K_{ow}) of 6 for the contaminant, using the relationship described by Thomann (1989). We assigned a value of 0.8 to E_f for our IBM simulations. Both E_d and E_x were set equal to 0.50, which was in accordance with the relationships described by Thomann (1989) when $\log_{10}$ (K_{ow}) for the contaminant was equal to 6. We tracked length, weight, and body burden of each individual lake trout in each simulation; PCB concentration was equal to body burden divided by weight and was expressed as milligrams PCB per kilogram wet weight of lake trout.

On the average, lake trout lose 1.9% of their PCB body burden during spawning (G. Noguchi, U.S. Fish and Wildlife Service, National Fisheries Research Center-Great Lakes, Ann Arbor, MI, unpubl. data). In our simulations, every fish at age 5 or older spawned on 28 October; each spawning fish lost 6.8% of its weight (Stewart et al. 1983) and lost 1.9% of its PCB body burden on that day. Apparently, lake trout lose a slightly lower fraction of their body burden from spawning compared with rainbow trout (*Oncorhynchus mykiss*); Niimi (1983) reported an average drop of 2.7% (assuming a negligible drop from spawning males, as observed for lake trout) in PCB body burden due to spawning in rainbow trout.

Barber et al. (1991) recently presented a new model for PCB accumulation in lake trout, and their model assumed that excretion of PCB through the gills is an important flux in the PCB budget of lake trout. Such an assumption was not supported by any laboratory studies on trout. Indeed, Weininger (1978) presented considerable experimental evidence to show that excretion through trout gills was negligible. We therefore chose the Thomann model, which yields results similar to those from the Weininger model, to represent the dynamics of lake trout PCB body burden.

Lake Michigan Data

Since 1970, the Michigan Department of Natural Resources has conducted surveys on the lake trout populations in the state's Lake Michigan waters (Rybicki and Keller 1978; Rybicki 1983). Lake trout were collected by gill nets during April and May; length, weight, and age were determined for each lake trout caught. A total of 12 725 lake trout were caught during this monitoring study from 1983 through 1990. A similar effort has been underway by the Wisconsin Department of Natural Resources for Wisconsin waters of Lake Michigan since 1977.

Prey fish populations in Lake Michigan have been monitored by the U.S. Fish and Wildlife Service since 1973 (Eck and Brown 1991). Relative abundances of forage fish were assessed by bottom trawls towed during the day in October and November at each of 10 index stations. Fish were identified to species, and individual lengths and weights were recorded. Temporal sequences over the growing season (April through November) for length–frequency distributions of prey fish in eastern Lake Michigan have been documented by Jude et al. (1981).

Jude et al. (1987) described lake trout diet for Lake Michigan, based on examining the stomach contents of lake trout sampled from eastern Lake Michigan from the years 1973 through 1982. Those fish were caught chiefly by gill nets. The University of Wisconsin Sea Grant Program sponsored a salmonid diet survey from 1982 through 1988 (Hagar 1984; Stewart and Ibarra 1991). Lake trout were caught by anglers fishing along the Wisconsin coast of Lake Michigan; lake trout stomach contents were collected and analyzed. Additionally, the U.S. Fish and Wildlife Service has occasionally examined the stomachs of lake trout caught in gill nets set off Port Washington, Wisconsin, and Saugatuck, Michigan (Eck and Wells 1983, 1986; G. Eck, unpubl. data).

The Wisconsin Department of Natural Resources received supplementary funds to intensively survey PCB contamination in Lake Michigan salmonids from March through October 1985 (Masnado 1987). Lake Michigan anglers agreed to donate a portion of their catch as samples for this study. Length and weight were measured for each of 125 lake trout. Then, lipid and PCB concentrations were determined in skins-on fillets from each of the 125 trout (see Masnado 1987 for details on laboratory analyses). Note that individual PCB congener concentrations were not measured; concentrations were reported for total PCBs.

PCB concentrations in the skins-on fillets should be "lipid-corrected" in order to better represent whole-body PCB concentrations (D. Endicott, U.S. Environmental Protection Agency, Environmental Research Laboratory, Grosse Ile, MI, pers. comm.). The manner of filleting can vary slightly from fish to fish. In a few cases, the fillet may have been cut so that its lipid content was not representative of the whole-body lipid content, and lipid correction allows for a more accurate representation of whole-body PCB concentration. We lipid-corrected our PCB concentration by multiplying the skins-on fillet PCB concentration by the ratio of expected lipid proportion of the whole-body weight and the lipid concentration (by weight) measured in the skins-on fillet. The expected lipid proportion of whole-body weight was taken from Rottiers and Tucker (1982).

Estimates of PCB concentrations in Lake Michigan prey fish, invertebrates, and water from 1975 through 1985 were needed to calibrate the IBM with PCB concentrations observed in Lake Michigan lake trout during 1985. Weininger (1978) estimated PCB concentrations in the various lake trout prey items in Lake Michigan for 1975. The Wisconsin Department of Natural Resources measured PCB concentration in Lake Michigan prey fish (alewife, smelt, sculpins, and bloater) from 1976 through 1985 (M. Miller, unpubl. data). Data were insufficient for determining seasonal trends in PCB concentrations in prey fish. Annual means of PCB concentration for each of the prey fish categories were used for simulation purposes (see Appendix 2). Additionally, PCB data were missing for certain combinations of year and prey categories; the missing concentrations were estimated by interpolation between known PCB concentrations for the particular prey category.

Few measurements of PCB concentration in Lake Michigan water have been made because of the difficulty of determining such low concentrations. Earlier determinations of these concentrations were probably higher than the actual levels because of the available technology for measuring these concentrations (Swackhamer and Armstrong 1987). Based on a relatively intensive survey of Lake Michigan during 1980, Swackhamer and Armstrong (1987) reported an average PCB concentration of 1.8 ng/L. We assumed that the PCB concentration in Lake Michigan water was constant at a level of 1.8 ng/L from 1975 through 1985, as recommended by D. L. Armstrong (Water Chemistry Program, University of Wisconsin, Madison, WI, pers. comm.). We used information from Weininger (1978) and Evans et al. (1991) to arrive at a schedule for PCB concentra-

tions in benthic invertebrates, including mysids and amphipods. *Mysis relicta* and *Diporeia* sp. are favorite prey items of young lake trout (Stewart et al. 1983).

The angler-caught lake trout sampled for PCB determinations by Masnado (1987) were from relatively shallow waters, and the IBM presented in this paper will be applied to these relatively shallow-dwelling fish. The unique subpopulation of lake trout from the midlake reef complex, where diets, temperature regimes, and growth rates are noticeably different from those of the rest of the Lake Michigan population (Miller and Holey 1992), was excluded from the model application.

We modeled total PCB concentrations, rather than concentrations of PCB congeners, because insufficient data were available to model PCB congeners. Modeling of specific congeners would have been more desirable than total PCBs because each congener has its own specific chemical properties and, consequently, its own properties associated with accumulation, such as assimilation efficiency, direct uptake rate through gills, and excretion rate. Some congeners are substantially more toxic than others (Niimi and Oliver 1989), and understanding bioaccumulation of the more toxic congeners is particularly important. Furthermore, when modeling total PCBs, the parameters associated with accumulation are aggregated across the range of congeners. Aggregation in model parameters should be kept to as low a level as possible (O'Neill and Rust 1979). Unfortunately, PCB congener analyses are costly and technically difficult (Safe et al. 1985). We hope to apply the IBM to specific congeners when these data become available.

IBM Application

First, the IBM was calibrated with growth data by matching observed with predicted length–frequency distributions for each of the age groups from ages 4 through 10. The "average" day of the year at which lake trout length–frequencies were observed was 1 May; thus, simulations were completed on that day of the appropriate simulation year. Encounter rates for each of the prey fish categories were adjusted to fit the model to the observed length–frequency distribution. Rough estimates of the encounter rates were based on diet survey data, but the rates were further adjusted by trial and error. Accuracy of the predicted length–frequency distribution was based on the difference between observed and predicted mean lengths of the cohort and on the sum of the squares of the differences between observed and predicted numbers of lake trout in each of the length intervals. Lake trout are not fully recruited to gill nets until age 4 (Rybicki and Keller 1978); hence, calibration with observed length–frequencies was performed for ages 4 through 10. The 500 simulated lake trout individuals were assigned initial lengths based on measurements of lake trout yearlings immediately prior to time of stocking (J. McClain, U.S. Fish and Wildlife Service, Iron River National Fish Hatchery, Iron River, WI, unpubl. data). Simulated lake trout were stocked on 15 June, which is a typical stocking day for Lake Michigan lake trout (J. McClain, unpubl. data).

The encounter submodel required considerably more computing time than use of a fixed proportion of maximum daily consumption. Hence, to reduce computing time, from time of first spawning to the end of the fifth simulation year, each lake trout consumed P_5 of its maximum daily consumption. Likewise, for the sixth, seventh, eighth, and ninth simulation years, each lake trout consumed P_6, P_7, P_8, and P_9 of its maximum daily consumption, respectively. The P_i's were adjusted to match the observed length–frequency distributions.

Next, the contaminant accumulation submodel of the IBM was calibrated using the 1985 Wisconsin Department of Natural Resources data set for PCB determinations (lipid-corrected values) in 125 individual lake trout. This calibration required a series of steps. The lake trout individuals used in the PCB determinations were not aged. Judging from the lengths, only one of the lake trout was age 2. Of the remaining 124 lake trout, we assumed that age was uniformly distributed between 5 and 8 and that the distribution tapered as age decreased from age 5 or increased from age 8. This assumption appeared reasonable because the researchers conducting the study attempted to bias their sampling toward both younger (ages 3 and 4) and trophy-sized fish (including ages 7 through 10) (R. Masnado, Wisconsin Department of Natural Resources, Madison, WI, pers. comm.) whereas peak catch for an unbiased sample would be expected at ages 5 and 6, when lake trout typically recruit to the sport fishery (R. Rybicki, pers. comm.). In an unbiased sample, few fish less than age 4 or older than age 7 would be expected. We chose a distribution that applied more weight to the extreme age groups than in an unbiased sample. We assumed that the PCB body burden of lake trout yearlings at time of stocking was zero because measurements on hatchery fish just prior to stocking revealed that PCB levels in these fish were below detection limits (J. Amrhein, Wisconsin Department of Natural Resources, Madison, WI, pers. comm.). Lake trout of age 11 and older were ignored in our simulations because these fish have been estimated to comprise less than 0.5% of the lake trout population that was vulnerable to fishing effort in Lake Michigan (R. Rybicki, unpubl. data).

To match the 125 observations (during 1985) for PCB concentration in Lake Michigan lake trout, we sampled 125 individuals from a simulated Lake Michigan lake trout population in the following manner. We simulated nine different cohorts corresponding to ages 2 through 10 in 1985. Each cohort was simulated until 15 July 1985, which was the "average" sampling day for the Masnado (1987) salmonid PCB sampling study. One lake trout individual (from a simulated cohort of 500 individuals) was randomly selected from the age-2 group. From the age-3 and age-4 groups, 6 and 12 individual lake trout were randomly selected, respectively. From each of the age-5 through age-8 cohorts, 22 individuals were picked. Finally, 12 and 6 individual lake trout were selected from the age-9 and age-10 groups, respectively.

Employment of our estimated schedule for PCB concentration in prey resulted in a simulated lake trout population with substantially less variance in PCB concentrations than that observed in the Lake Michigan population. Furthermore, when PCB assimilation efficiency from food, E_f, was increased to 1.0, the maximum PCB concentration in the simulated age-10 lake trout population was 11 mg/kg. Yet, PCB concentrations in several lake trout were observed at levels greater than 16 mg/kg. A refinement in the IBM was needed to reproduce the variation expressed in the observed PCB concentrations. We divided the simulated cohort of 500 lake trout into five groups of 100 fish. The PCB concentration for each prey item consumed by fish in group 1 was set equal to $(100 - x)$ % of the scheduled concentration, group-2 prey items contained PCB at concentrations of $(100 - (x/2))$ % of the scheduled concentrations, lake trout in group 3 were exposed to the scheduled concentrations, group-4 prey were at concentrations of $(100 + (x/2))$ % of the scheduled concentrations, and group-5 prey items were at $(100 + x)$ % of scheduled concentration. We examined model accuracy when the prey PCB variation factor, x, was equal to zero (no variation between the five groups), 20,

TABLE 1. Nominal values of parameters used in uncertainty analysis of individual-based model for accumulation of PCBs by lake trout in Lake Michigan.

Parameter	Description	Parameter value
C	Multiplier of the C_{max} term in consumption component of the bioenergetics submodel (dimensionless)	1.0
R	Multiplier of the loss terms (respiration, excretion, egestion, and specific dynamic action) of the bioenergetics submodel (dimensionless)	1.0
λ	Multiplier of the prey encounter rates listed in Appendix 1 (dimensionless)	1.0
γ	Maximum ratio of prey fish length to lake trout length at which the prey fish was still vulnerable to predation by the lake trout (dimensionless)	0.4
E_d	Efficiency of direct uptake of PCBs by gills of lake trout (dimensionless)	0.5
E_x	Efficiency of excretion of PCBs by lake trout (dimensionless)	0.5
E_f	Efficiency of assimilation of PCBs from food consumed by lake trout (dimensionless)	0.8
W	Concentration of PCBs in Lake Michigan water (ng/L)	1.8
F	Multiplier of concentrations of PCBs in food of lake trout (dimensionless)	1.0

40, and 60. Accuracy was evaluated based on the difference between the observed and predicted regression lines of $\log_e$ PCB concentration on total length of lake trout and based on the difference between the observed and predicted variances about the regression lines for $\log_e$ PCB concentration against length.

Uncertainty Analysis

Sensitivity of the IBM to parameter values was investigated using the same type of uncertainty analysis employed by Madenjian and Carpenter (1991). We were chiefly interested in the relative importance of the main components of the three submodels (bioenergetics, predator–prey encounter, and PCB accumulation), and therefore, some of the parameters were actually scalars (each scalar equal to a value of 1 during nominal simulations) of a set of parameters or of an expression of model terms (Table 1). The encounter rate scalar, λ, was a multiplier of the set of encounter rates presented in Appendix 1, C was a multiplier of consumption term, CONS, of the bioenergetics model described by equation 1, R was a multiplier of the sum of RESP, EG, EX, and SDA shown in equation 1, and F was a multiplier of the prey PCB concentrations presented in Appendix 2.

The relative partial sum of squares (RPSS) technique involved treating the model input parameters of interest as random variables with normal distributions; the mean of the distribution was the nominal value of the parameter. Then, 100 combinations of parameters were selected using Latin hypercube sampling (Iman and Conover 1980). For sensitivity analysis, the coefficients of variation (CVs) of 2% defined the normal distribution of each input parameter. CVs of 20% were used in error analysis to better represent the degree of uncertainty associated with parameters in ecological models. The output variables included the mean PCB concentration for the cohort of 500 simulated lake trout and the number of contaminated fish (NCF); NCF is defined as the number of fish, in a cohort of 500 fish, with a PCB concentration ≥2 mg/kg. For uncertainty analysis, a cohort of 500 lake trout was simulated from time of stocking, 15 June 1981, to 15 July 1985 using a PCB variation factor of 40. Lake trout are first fully recruited to the Lake Michigan sport fishery at age 5, after 4 yr in the lake (Rybicki and Keller 1978). RPSS represents the percentage of variation in the output variable that is explained by the input parameter.

Additional Simulations

To demonstrate that the predator–prey encounter submodel was an important contributor to size variation within a lake trout cohort, the following simulation was conducted. We simulated a cohort of 500 lake trout from time of stocking to time of first spawning (during simulation year 5) without including the predator–prey encounter submodel. Rather, each lake trout received a fixed proportion of its maximum daily consumption for each day of the simulation. The range of trout lengths for this simulated cohort was compared with that for a simulation run in which the predator–prey encounter submodel was included in the IBM.

To investigate the effect of stochastic variation in prey PCB concentrations on variation in lake trout PCB concentrations within a cohort, we performed the following simulation. We assumed that PCB concentration for a particular prey category was normally distributed with mean equal to the nominal PCB concentration and standard deviation equal to 20% of the nominal PCB concentration. For each prey item encountered, a PCB concentration was randomly chosen from the normal distribution for that prey categry. Results from this simulation were compared with a simulation run in which there was no stochastic variation in prey PCB concentration. The lake trout cohort was simulated from time of stocking through 15 July of the fifth simulation year for both simulation runs. For both runs, the prey PCB variation factor was equal to zero.

Several additional simulations were performed to address influences on the average PCB concentration of the lake trout cohort by the following factors: (1) PCB loss from the body burden due to lake trout spawning, (2) lake trout lipid concentration (see equation 5), and (3) a diet composed of only adult alewife for lake trout. Spawning loss was assessed by doubling the nominal value of 1.9% loss in body burden and then simulating a lake trout cohort to age 6 and a second cohort to age 10. Average PCB concentration in the cohort at the end of the simulation was compared with the nominal simulation run. Similarly, the nominal lipid concentration value of 15.5% was doubled, and corresponding simulations (ages 6 and 10) were run, in order to investigate the effect of lipid concentration on lake trout PCB concentration. To assess the effect of an exclusively alewife diet, we performed a simulation in which a diet of strictly adult alewife was imposed on each lake trout from the time of first spawning, when the fish had spent about 4.35 yr in the lake, through a 9-yr duration in the lake. Adult alewife exhibited the highest PCB concentrations of all the prey fish categories.

Results

Following the encounter rate schedule presented in Appendix 1, observed and predicted length–frequency distributions were in reasonably good agreement for ages 4 through 10 (Fig. 3). The prey–predator encounter submodel was employed until 28 October of the fifth simulation year. After

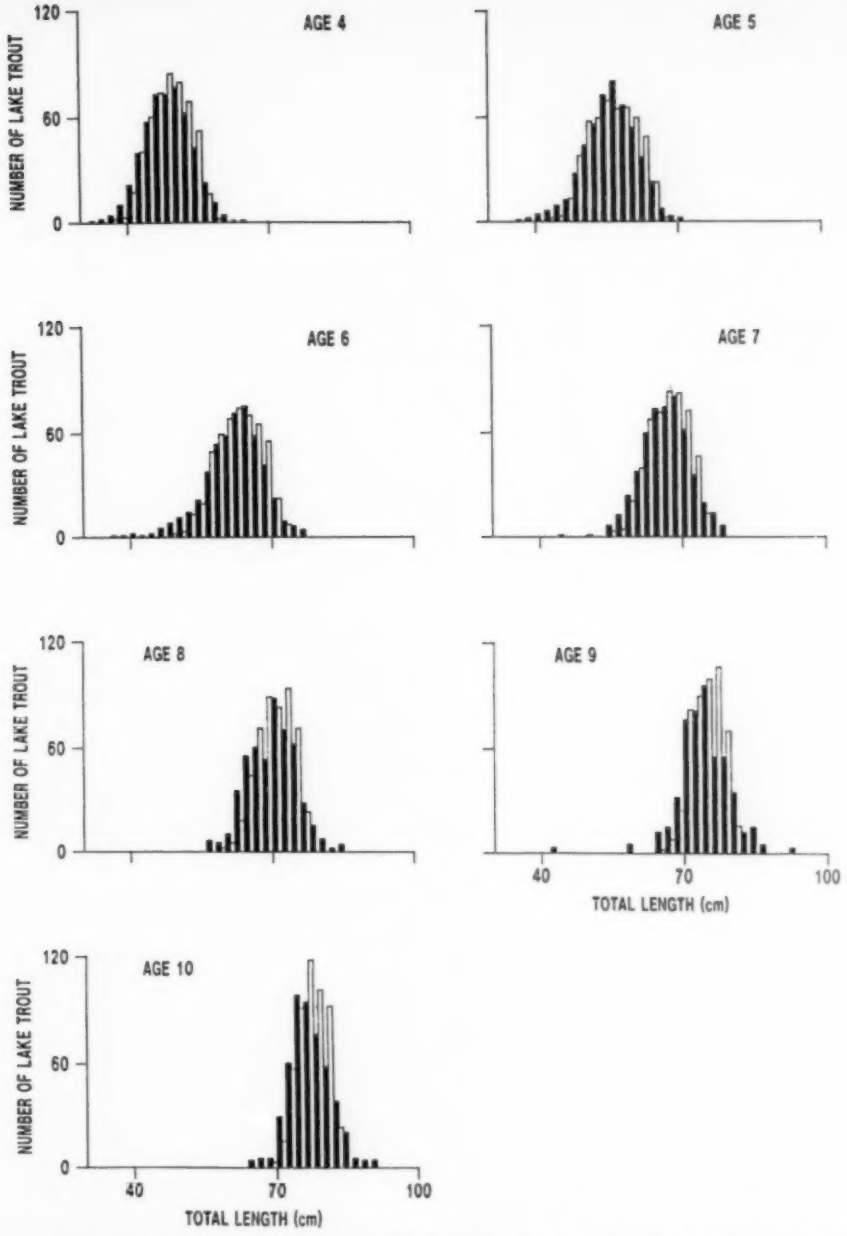

FIG. 3. Observed and predicted length–frequency distributions for ages 4 through 10 lake trout from Lake Michigan. Observed (black bars) distributions were based on lake trout caught in Lake Michigan from 1983 through 1990 (R. Rybicki, unpubl. data). Predictions (white bars) were based on an IBM for lake trout growth. For each age group, a cohort of 500 observed fish was compared with a cohort of 500 simulated fish.

that time, use of the encounter submodel did not provide substantially better accuracy in predicting the observed length–frequency distributions than simply using a constant P value, the proportion of maximum daily consumption, for each individual of the cohort (see Appendix 1 for P_i values).

When the PCB variation factor, x, was equal to zero, the observed and predicted regression lines of natural logarithm ($\log_e$) of PCB concentration against lake trout total length were significantly different (Table 2). Variance about the predicted linear relationship between $\log_e$ PCB concentration and length was significantly less than the observed variance (Table 2; Fig. 4). Observed and predicted variances about the regression lines were in agreement when x was increased to 40 (Table 2; Fig. 5). In other words, when the extreme groups in a particular

TABLE 2. Comparison of observed and predicted regression lines of the natural logarithm of lake trout PCB concentration as a linear function of total length. Observations were from lake trout captured by anglers in Wisconsin waters of Lake Michigan during 1985. Predictions were based on an individual-based model for PCB accumulation in lake trout from Lake Michigan. Four sets of predictions were made corresponding to x, the prey PCB variation factor (see IBM Application section), equal to 0, 20, 40, and 60. Results from F-tests for equality of regression lines and equality of variances about the regression lines are presented. *Significant difference ($\alpha = 0.05$).

	Attained significance level of F-test	
Prey PCB variation factor	Equality of regression lines	Equality of variances about regression line
0	0.000350*	0.000000*
20	0.004877*	0.000002*
40	0.160548	0.454886
60	0.677812	0.000003*

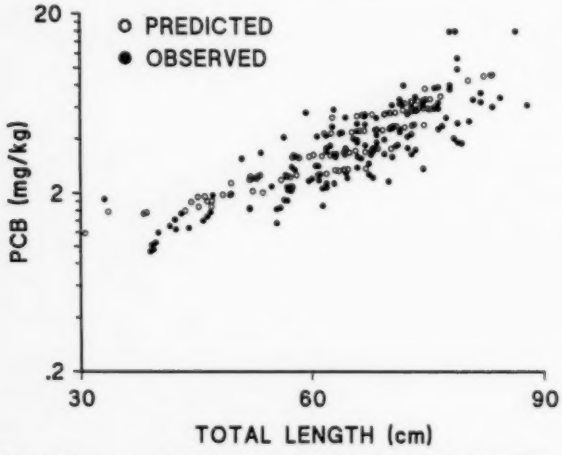

FIG. 4. Observed and predicted whole-fish PCB concentrations versus total length of lake trout from Lake Michigan in 1985. Observations were from lake trout sampled by the Wisconsin Department of Natural Resources (M. Miller, unpubl. data). Predictions were based on an IBM for accumulation of PCBs by lake trout, using a prey PCB variation factor equal to 0 (see IBM Application section).

lake trout age cohort were subjected to a 40% deviation from the scheduled PCB, predicted and observed variances about the respective regression lines were closely matched (Table 2). Predicted variance about the regression line significantly exceeded the observed variance when x was further increased to 60.

Sensitivity analysis indicated that food assimilation efficiency of PCB and the respiration component (R) of the bioenergetics submodel were the major factors explaining mean PCB and NCF ($\geq$2 mg PCB/kg) for lake trout recruiting to the Lake Michigan sport fishery (Fig. 6). Food PCB concentration (F) was paramount to determining mean PCB and NCF in the error analysis. The consumption bioenergetics component (C), prey encounter rate (λ), prey–predator length vulnerability ratio (γ), and efficiencies of PCB direct uptake and excretion (E_d and E_x, respectively) had relatively little impact on mean PCB or NCF in the lake trout cohort. The IBM behaved almost linearly over the small perturbations imposed upon the parameters for sensitivity analysis; R^2 values for the regression of mean PCB or NCF predicted from the simulation trial against the set

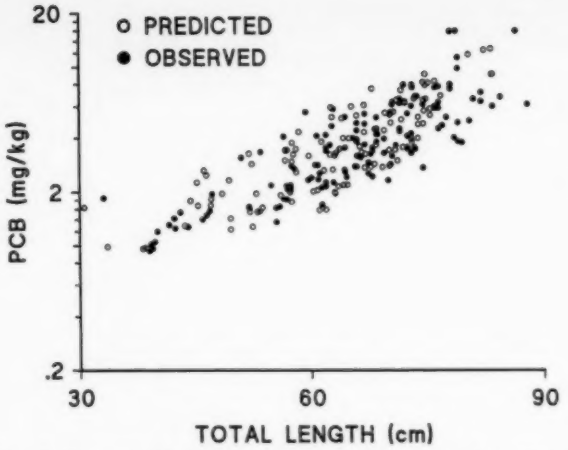

FIG. 5. Observed and predicted whole-fish PCB concentrations versus total length of lake trout from Lake Michigan in 1985. Observations were from lake trout sampled by the Wisconsin Department of Natural Resources (M. Miller, unpubl. data). Predictions were based on an IBM for accumulation of PCBs by lake trout, using a prey PCB variation factor equal to 40 (see IBM Application section).

of simulation model parameters ranged from 0.91 to 0.97. Corresponding R^2 values for error analysis ranged from 0.78 to 0.86.

Results from the additional simulations revealed that the predator–prey encounter submodel was an important contributor to size variation within a simulated lake trout cohort. When the predator–prey encounter submodel was omitted from the model, lake trout lengths were narrowly clumped from 560 to 604 mm at the completion of the simulation run whereas lengths ranged from 442 to 680 mm when the encounter submodel was employed. Use of the encounter submodel yielded a much more realistic representation of range in lake trout lengths than that for a model lacking the encounter submodel. For both simulations, final mean length of the cohort was 579 mm.

Allowing for stochastic variation within the prey PCB concentrations yielded the same results as the simulation in which no stochastic variation was allowed. This interesting modeling result can be explained by the following reasoning. On one day, an individual lake trout may consume an adult alewife with below-average PCB concentration, but then the same lake trout may consume an adult alewife with above-average PCB concentration the following day. Thus, over the course of several growing seasons, the simulated lake trout PCB concentrations were nearly identical whether or not stochastic variation in prey PCB concentration was incorporated in the model.

Other results from the additional simulations showed that PCB spawning loss, lake trout lipid concentration, and a specialized adult alewife diet had minor impacts on the average PCB concentration of a simulated lake trout cohort. Doubling the fraction of PCB body burden lost during spawning led to only 1.0 and 5.3% decreases in average lake trout PCB concentration at the end of the age-6 and age-10 simulation runs, respectively. The effect of lake trout lipid concentration was of even less importance. Doubling the value of lake trout lipid concentration, ρ, resulted in 0.02 and 0.04% increases in average lake trout PCB concentration at the end of the age-6 and age-10 simulation runs, respectively. An all-adult alewife diet, after age 5, yielded an age-10 simulated lake trout cohort with

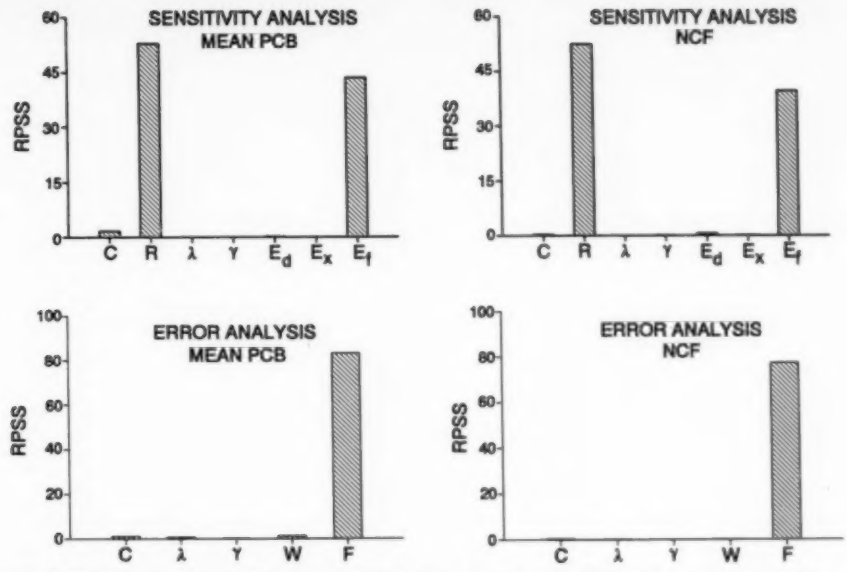

FIG. 6. Relative partial sum of squares (RPSS) for parameters of the IBM for accumulation of PCBs by lake trout in Lake Michigan. "MEAN PCB" refers to the mean whole-fish PCB concentration (mg/kg) of the simulated population at age 5, and "NCF" refers to the number of contaminated fish (fish with PCB concentration ≥2 mg/kg) within the simulated population of 500 fish at age 5. Symbols below the bars are defined in Table 1. For details on sensitivity and error analyses, see Uncertainty Analysis section.

only a 4.9% greater average PCB concentration than that yielded from the nominal simulation run.

Discussion

The first objective of this study was to determine if the IBM approach could explain the size variability observed within the various age cohorts of Lake Michigan lake trout. The reasonably good agreement between observed and predicted length–frequency distributions demonstrated that the IBM was capable of explaining the size variability (Fig. 3). The next objective of the study was to determine whether the IBM could explain the variation in Lake Michigan lake trout PCB concentrations when only age and size differences between individual lake trout were included in the model. Our findings showed that the variability in PCB concentrations within the lake trout population was not fully accounted for when only these sources of variation were included in the IBM (Table 2; Fig. 4). Therefore, at least one other additional source of variation was needed to account for the observed variation in lake trout PCB concentrations.

Neither variability in model parameters nor stochastic variation in prey PCB concentrations accounted for all of the remaining variability in lake trout PCB concentrations. Sensitivity analysis showed that simulated lake trout PCB concentrations were very sensitive to the assimilation efficiency, E_f (Fig. 6). Yet even if lake trout assimilated PCBs from food with 100% efficiency, the model could not explain the higher PCB concentrations observed in Lake Michigan lake trout unless the trout were subjected to greater-than-nominal PCB concentrations in the prey fish. Variability in the percent loss of body burden from spawning or the lipid content parameters contributed little to modeled variation in PCB concentrations. The relatively small impact of lake trout lipid concentration on PCB accumulation in lake trout, as illustrated by the additional IBM simulation trials, suggested that the high correlation between lipid content and PCB concentration observed in the Lake Michigan lake trout population may be an artifact of the increase in PCB concentration with age. Both lipid concentration and length are correlated with lake trout PCB concentration, although length is the stronger correlate (M. Miller, unpubl. data). Lipid content increases with age or length (Rottiers and Tucker 1982). Results from an additional simulation showed that stochastic variation in prey PCB concentrations had a negligible effect on lake trout PCB concentrations predicted by the IBM.

The sensitivity analysis indicated that the respiration component of the bioenergetics submodel exerted a strong effect on PCB accumulation in lake trout. Mean PCB concentration increased with respiration. Increasing respiration rate, while holding the consumption component constant, is the equivalent of decreasing gross growth efficiency. Gross growth efficiency is that fraction of the ingested food that is converted to fish growth. The IBM specifically takes into account differences in gross growth efficiencies between individuals of the cohort based on size differences.

If the concentration of PCB in the prey varied among different groups of lake trout constituting the Lake Michigan population, then the model accurately predicted variation in PCB concentrations of lake trout caught throughout the lake (Fig. 5). In theory, various groups of lake trout in Lake Michigan may be segregated due to spatial, behavioral, or physiological (i.e. chemical assimilation efficiency, E_f) barriers. There is some evidence to corroborate the notion that spatially distinct stocks of lake trout can exhibit different levels of PCB contamination. Swackhamer and Armstrong (1988) have shown that certain

areas of inshore Lake Michigan were "hot spots" or areas of high concentration for PCBs in surficial sediments. These areas were in the vicinities of major point sources of PCBs to Lake Michigan and included Green Bay, especially at the mouth of the Fox River, and Waukegan Harbor, Illinois, located on the southwestern shore of Lake Michigan, as well as several other sites in Michigan and Illinois. Based on the extraordinarily high PCB levels in sediments and fish (Sheffy 1980), the Sheboygan River may have been a major point source of PCBs to Lake Michigan fishes during the late 1970's. Prey fish aggregations which had more exposure to "hot spots" should show higher PCB concentrations than prey fish with a "cleaner" history of movement through the lake. PCBs from sediments may be transferred to lake trout via the benthic invertebrate – prey fish – lake trout food chain (Nalepa and Landrum 1988). Variation in PCB concentration in Great Lakes prey fish species has not been well studied. Significant differences in mean PCB concentrations of spottail shiner (*Notropis hudsonius*) YOY have been demonstrated between different sites on Lake Ontario (Suns and Rees 1978; Suns et al. 1991) and on Lake Erie (Suns and Rees 1978), although spottail shiners are rarely eaten by Lake Michigan lake trout (Jude et al. 1987). The dynamics of "hot spots" is unknown, but it seems likely that locations of these areas within a lake may change with time in response to movements of sediments.

Any explanation for the variation in both prey and lake trout PCB concentrations should also address the movements and migrations of both lake trout and prey species within Lake Michigan. Several tagging studies in Lake Superior have shown that lake trout exhibit a propensity toward the region of the lake in which they were either stocked or spawned (Rahrer 1968; Swanson 1974; Swanson and Swedberg 1980). Furthermore, lake trout tend to return to the very site at which they were stocked or spawned for a period of several months each year (Swanson and Swedberg 1980). Similar movement patterns were observed for lake trout planted nearshore in Lake Michigan (M. Holey, Wisconsin Department of Natural Resources, Sturgeon Bay, WI, pers. comm.). Miller and Holey (1992) concluded that fin-clipped lake trout stocked on the midlake reef complex in Lake Michigan displayed site fidelity. In summary, there is evidence to support the contention that the lake trout population in Lake Michigan is composed of several spatially distinct subpopulations. The movements of alewife and smelt within Lake Michigan are poorly understood. Unless the movements of these prey fish are completely random and throughout the entire lake, then it is plausible that certain aggregations of alewife and smelt contain higher PCB levels than other aggregations. Finally, lake trout subpopulations closer to "hot spots" are probably subjected to higher PCB concentrations in their prey than lake trout stocks from "cleaner" areas.

Swackhamer and Armstrong (1988) concluded that the point source of PCBs originating from Waukegan Harbor influenced PCB burden of southern Lake Michigan sediments within the nearshore region of the harbor. We therefore may expect that prey fish tending toward the nearshore regions of the lake exhibit higher PCB levels than offshore prey fish. The Wisconsin Department of Natural Resources prey fish PCB data set was inadequate to address this difference.

Error analysis of the IBM presented in our study corroborated the hypothesis that variation in prey PCB concentration was an important contributor to variation in Lake Michigan lake trout PCB concentration. PCB concentration in the lake trout prey was the major determinant of mean PCB concentration of the trout. It is reasonable, then, to expect that variance in the major determinant of a response variable would be the most likely candidate to explain variance in the response variable. An alternative hypothesis, that the assimilation efficiency of PCB from food (E_f) varied between individual lake trout, is insufficient to explain the variability in lake trout PCB concentration. The nominal value of E_f for our simulations was 0.8, which is within 25% of its maximum allowable value of 1.0. Yet a 40% variation band in the prey PCB schedule had to be imposed to accurately portray the lakewide variation in lake trout PCB concentration.

Another explanation for differences between PCB concentrations of lake trout is that different individuals within the Lake Michigan population showed different feeding preferences. The favored prey fish may have varied between individuals. No study has addressed this question, and such an investigation would require analysis of food habits of individual fish over an extended period of time. Lake trout diet may have varied slightly between regions of the lake. Eck and Wells (1983) found that smelt constituted a higher proportion of the lake trout diet during ages 2 through 5 on the Wisconsin side of Lake Michigan than on the Michigan side. However, alewife was the dominant prey fish (comprising more than 80% of the diet) for age 6 and older lake trout from both sides of the lake. Supplementary IBM simulations indicated that even if an individual lake trout consumed only adult alewife (the prey fish category with the highest PCB concentration) over the entire portion of its lifetime that it was capable of feeding on this prey item, it would not attain the maximum PCB levels observed in Lake Michigan lake trout unless the trout were fed alewife with higher-than-average PCB concentrations. Undoubtedly, differences in lake trout diet composition between individual fish were responsible for some of the variation in lake trout PCB concentration, but diet differences alone could not explain all of the variability in lake trout PCB concentration.

Our simulation study has suggested that spatial variation in PCB concentration within the food chain may be an important component of the variation in lake trout PCB concentrations in Lake Michigan; two implications can be derived from this postulation. To estimate mean PCB concentration in Lake Michigan lake trout, samples should include a wide range in lake trout length, and trout from regions near "hot spots" and from cleaner areas should be sampled. We would expect that lake trout near "hot spots" are subjected to prey with higher PCB concentrations than lake trout from "cleaner" waters. As mentioned earlier, the spatial variation in prey fish PCB concentrations has been poorly studied.

Our simulation results also indicated that if individuals of a particular salmonine population traveled throughout Lake Michigan rather than confine themselves to local environments, then less variability in PCB concentration for a given age would be expected than that for lake trout. If each individual traveled throughout the entire lake, than each fish in the lake would be subjected to the same gamut of prey PCB concentrations in the lake. Consequently, any individual of a particular age would receive roughly the same exposure as any other individual of equal age. Chinook salmon (*Oncorhynchus tshawytscha*) is the most mobile salmonine species in Lake Michigan (T. J. Lychwick, Wisconsin Department of Natural Resources, Green Bay, WI, pers. comm.). The diet of chinook salmon of age 2 or older in Lake Michigan is dominated by alewife (Stewart and Ibarra 1991). However, PCB observations for Lake Michigan chinook salmon suggest that some individuals confine themselves to the northern half of the lake, including the northern part of the main basin and Green Bay. Green Bay alewife show higher PCB con-

centrations than alewife from the main basin of Lake Michigan (M. Miller, unpubl. data). Salmon collected in Green Bay and the northern section of the main basin exhibited significantly higher PCB levels than salmon from the southern section of the main basin (Masnado 1987). Hence, it appears that spatial variability in prey fish PCB concentrations plays an important role in determining variability of PCB concentration in even the most mobile salmonine species in the lake.

The hypothesis that PCB concentration varies among subpopulations of each category of prey fish throughout Lake Michigan could be tested by sampling prey fish from a diverse array of locations within the lake over as short a time period as possible and then determining PCB concentration for each prey fish category from each site. Sampling locations should be planned to contrast (1) "hot spots" and cleaner areas, (2) northern and southern Lake Michigan, and (3) nearshore and offshore environments. The watershed of the southern portion of Lake Michigan is considerably more economically developed than that of the northern portion. An east–west comparison may also be very informative because zones of heavy sediment deposition in the southern part of the lake are closer to the Michigan shore than the Wisconsin or Illinois shore (Swackhamer and Armstrong 1988).

The individual-based modeling approach used in this study proved to be a valuable research tool because it enabled us to explicitly include diet differences between individual lake trout of the same cohort in the simulation model. Having demonstrated that within-cohort variation and between-cohort variation were insufficient to explain the lakewide variation in lake trout PCB, the IBM was easily modified to include variation in prey PCB schedules. Ultimately, IBM applications could contribute toward (1) determination of stocking scenarios that minimize PCB concentration in lake trout, (2) development of an aggregated ecosystem model for PCB flux through the Lake Michigan food web, and (3) development of more realistic advisories and regulations regarding consumption of fish from Lake Michigan. More specifically, the IBM can be used to simulate the accumulation of PCBs by a lake trout cohort stocked into Lake Michigan over various combinations of size at stocking and timing of stocking. Then, the stocking strategy that minimizes PCB accumulation in the cohort can be identified. Additionally, patterns that emerged from application of the IBM to PCB accumulation in different salmonine species could be incorporated into a food web model for the entire Lake Michigan system. Such a model could be used to manage the stocking of salmonines in order to keep PCB concentrations in the fish to a minimum. The food web model could also be used to investigate the response of chemical accumulation in salmonines to changes in water and sediment concentrations. Finally, the IBM could be used in risk assessment of organochlorine contamination of Great Lakes fishes. Risk assessment requires an estimate of the probability of a contaminated fish. The IBM can explicitly yield an estimate of the probability that a fish in the population will exceed a specified level of contamination, given a particular contaminant schedule for prey.

Acknowledgments

Thanks to J. Breck and J. Kitchell for their reviews of this manuscript. We are grateful to D. Jude (University of Michigan) for providing lake trout food habit data, G. Noguchi and M. Mac (U.S.F.W.S., National Fisheries Research Center - Great Lakes, Ann Arbor, MI) for analysis of PCB loss from spawning lake trout, R. Rybicki and J. Gapczynski (Michigan Department of Natural Resources) for lake trout length–frequency distributions by age, J. McClain (U.S.F.W.S., Iron River National Hatchery, Iron River, WI) for providing length–frequency distributions of yearling lake trout at stocking time, and M. Hansen (now with U.S.F.W.S., Ann Arbor, MI), M. Holey, and P. Peeters (Wisconsin Department of Natural Resources) for lake trout and prey fish length–frequency distributions. This study was greatly strengthened by conversations with J. Amrhein, A. Andren, D. Armstrong, J. Brazner, P. Cook, R. Elliott, D. Endicott, J. Hartig, A. Hasler, M. Holey, R. Horrall, D. Jude, M. Mac, S. Marcquenski, R. Masnado, G. Noguchi, and P. Peeters. T. Martin assisted with preparation of computer-generated figures. G. Chipman assisted in ordering and installing the computer equipment. This work was funded by the University of Wisconsin Sea Grant Institute under grants from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, and from the State of Wisconsin. Federal grant NA90AA-D-SG469, project R/MW-41.

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Appendices

APPENDIX 1. Average prey encounter rates (number of encounters per day) for an individual lake trout used in individual-based model simulations of PCB accumulation by lake trout in Lake Michigan. Rough estimates of the prey encounter rates were based on diet surveys by Jude et al. (1987), G. Eck (unpubl. data), and M. Miller (unpubl. data). Prey encounter rates were then adjusted so that predicted length–frequency distributions over the various lake trout ages matched the observed length–frequency distributions. YOY = young-of-the-year, YRL = yearling, AD = adult. After 28 October during simulation year 5, the Stewart et al. (1983) (updated by Stewart and Ibarra 1991) bioenergetics model, using *P* values, was applied to each individual in the population. See Results section for details.

Simulation year	Season	Sculpins	Rainbow smelt			Alewife			Bloater
			YOY	YRL	AD	YOY	YRL	AD	YOY
1	June–Oct.	2.0	0.2	1.0	0	0	0.2	0	0
1	Nov.	2.0	3.0	1.0	0	1.0	0.2	0	0
1	Dec.	2.0	2.5	1.0	0	1.0	0.2	0	0
1	Jan–Apr.	2.0	0	1.0	0	0	0.6	0	0
1	May	1.0	0	0.7	0	0	0.5	0	0
2	June–Oct.	0.6	0	0.7	2.0	0	0.6	0.5	0
2	Nov.	0.6	1.0	0.7	1.0	1.0	0.5	0	0
2	Dec.	0.6	0	0.7	1.0	0	0.5	0	0
2	Jan.–Apr.	1.0	0	0.7	1.0	0.5	0.5	0	0
2	May	0.6	0	0.5	2.0	0	0.5	0.5	0
3	June–Oct.	0.5	0	0.5	3.0	0	0.7	1.0	0
3	Nov.	0.5	1.0	0.5	2.0	1.0	0.7	0	0
3	Dec.	0.5	0	0.5	1.0	0	0.7	0	0
3	Jan.–Apr.	1.0	0	0.5	2.0	0	0.7	1.0	0
3	May	0.2	0	0.2	1.0	0	0.4	1.7	0
4	June–Oct.	0.2	0	0.2	1.0	0	0.4	1.7	0
4	Nov.	0.2	1.0	0.2	1.0	1.0	0.4	0	1.0
4	Dec.	0.2	0	0.2	1.0	0	0.4	0	0
4	Jan.–Apr.	1.0	0	0.2	1.0	0	0.4	1.7	0
4	May	0.2	0	0.2	1.0	0	0.4	1.7	0
5	June–Oct.	0.2	0	0.2	1.0	0	0.4	0.9	0
	P value								
5	0.5350								
6	0.4650								
7	0.4570								
8	0.5200								
9	0.4500								

APPENDIX 2. Schedule for PCB concentration in lake trout prey used in individual-based model simulation of PCB accumulation by Lake Michigan lake trout. The schedule was based on observations from Weininger (1978) and from the Wisconsin Department of Natural Resources (M. Miller, unpubl. data). For most years, yearling alewife PCB concentrations were not available; the PCB concentrations for unknown yearling alewife were adjusted so that predicted lake trout PCB concentrations would match the PCB concentrations observed in Lake Michigan lake trout in 1985. All concentrations are reported in mg/kg. YOY = young-of-the year, YRL = yearling, AD = adult.

Year	Invertebrates	Sculpins	Rainbow smelt			Alewife			Bloater
			YOY	YRL	AD	YOY	YRL	AD	YOY
1976	0.8	1.5	0.8	1.3	1.7	1.0	1.0	4.5	1.0
1977	0.6	1.3	0.7	1.1	1.4	0.9	0.9	3.6	0.9
1978	0.4	1.1	0.6	0.9	1.1	0.8	0.8	2.7	0.8
1979	0.3	0.9	0.5	0.7	0.8	0.7	0.7	1.8	0.7
1980	0.2	0.8	0.4	0.5	0.6	0.6	0.6	1.65	0.6
1981	0.15	0.8	0.35	0.4	0.5	0.5	0.5	1.5	0.5
1982	0.15	0.7	0.3	0.35	0.4	0.5	0.5	1.35	0.5
1983	0.1	0.6	0.3	0.35	0.4	0.5	0.5	1.2	0.5
1984	0.1	0.5	0.25	0.3	0.35	0.4	0.4	1.1	0.4
1985	0.1	0.5	0.25	0.3	0.35	0.4	0.4	1.0	0.4

Production of Fish Populations in Lakes[1]

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Downing, J. A., and C. Plante. 1993. Production of fish populations in lakes. Can. J. Fish. Aquat. Sci. 50: 110–120.

Biological production estimates of 100 fish populations from 38 lakes worldwide were gathered from the literature. The relationship between the annual production of fish populations (*P*, kilograms per hectare per year), annual mean standing biomass (*B*, kilograms per hectare), and maximum individual body mass (*W*, grams) was approximately $\log_{10}P = 0.32 + 0.94 \log_{10}B - 0.17 \log_{10}W$ ($R^2 = 0.84$). This relationship is similar to one observed for lotic invertebrate populations and shows that *P* declines with *W*. Major axis regression indicated that the *P*/*B*:*W* relationship had an exponent similar to that predicted by allometric theory. The residuals from this multivariate equation suggest that fish production is positively correlated with temperature, lake phosphorus concentration, chlorophyll *a* concentration, primary production, and with pH. The results suggest a general bottom-up control of lake ecosystem components. The morphoedaphic index is not a good predictor of the production of fish populations. Assuming that sustainable yield is about 10% of production, sustainable yield would be less than 15% of the standing biomass for the majority of fish populations analyzed. Exploited populations were found to be about 70% more productive, on average, than unexploited populations of the same standing biomass and body-mass.

On a relevé, dans les ouvrages publiés, des estimations de la production de 100 populations de poissons vivant dans 38 différents lacs à travers le monde. La relation entre la production annuelle des populations de poissons (*P*, kilogrammes par hectare par année) la biomasse annuelle moyenne (*B*, kilogrammes par hectare) et la masse maximale individuelle (*W*, grammes) est décrite par l'équation suivante : $\log_{10}P = 0{,}32 + 0{,}94 \log_{10}B - 0{,}17 \log_{10}W$ ($R^2 = 0{,}84$). Cette équation est similaire à celle obtenue pour les populations d'invertébrés lacustres. Elle démontre que *P* diminue avec *W*. L'axe majeur entre *P*/*B* et *W* donne un exposant similaire a celui prédit par la théorie allométrique. Les résidus de cette équation suggèrent que la production est positivement corrélée avec la température, la concentration en phosphore total, la concentration en chlorophylle *a*, la production primaire et avec le pH. Les résultats suggèrent un contrôle des composantes de l'écosystème par la disponibilité en nutriments. L'indice morpho-édaphique n'est pas un bon prédicteur de la production des populations de poissons. En supposant que le rendement de pêche équilibré soit 10 % de la production annuelle d'une population, ce rendement équilibré serait moins que 15 % de la biomasse présente pour la majorité des populations analysées. On trouve que les populations exploitées sont d'environ 70% plus productives que les populations non-exploitées.

Received February 7, 1992
Accepted July 8, 1992
(JB398)

Reçu le 7 février 1992
Accepté le 8 juillet 1992

Freshwater fish populations are of major ecological and economic importance. Fish play a major ecological role in structuring the benthic and zooplanktonic invertebrate communities. For example, recent research shows that the presence and relative abundance of fish populations with various trophic characteristics can alter zooplankton size structure and abundance (Benndorf et al. 1984; McQueen et al. 1986), nutrient cycling (Bartell and Kitchell 1978; Mazumder et al. 1988), algal community structure (Mazumder et al. 1988), water clarity (McQueen et al. 1990), and even the heat budgets of lakes (Mazumder et al. 1990). Economically, sport and commercial freshwater fisheries generate about $2 billion annually in Canada alone (Pearse 1988) and sportfishing's social value lies in its role as a frequently enjoyed recreational experience.

At equilibrium, unexploited fish stocks produce exactly enough biomass to balance natural mortality. One of the principal aims of contemporary fisheries management is to balance the rate of renewal of fish populations as closely as possible with the sum of natural and fishing mortality (e.g. Schaefer 1968). The renewal of fish biomass is provided by production, which is the "amount of tissue elaborated per unit time per unit area, regardless of its fate" (Clarke 1946). It is thus of interest to fisheries ecologists to know how fish production varies among ecosystems and populations. A first step toward this goal is to determine which characteristics of ecosystems have the greatest impact on this rate of renewal.

Until recently, confusion in this field was fostered by a proliferation of models using differing indices of fish production (reviewed by Downing et al. 1990). Not surprisingly, these models often yielded widely variable, and sometimes contradictory, results, probably in part because models dealt with such disparate measures of fish production as commercial fish yield, sportfishing yield, long- or short-term average catch, net growth increment, fish catch, creel censuses, etc. (Downing et al. 1990). Variables that have been found to be correlated with indices of "fish production" in these studies are lake area, mean depth, alkalinity, total dissolved solids, total nitrogen, total phosphorus, or chlorophyll *a* concentration of the water column, primary productivity, benthos abundance, air temperature, and fishing effort. In a recent study of fish community

[1]This is publication No. 390 of the Groupe d'Ecologie des Eaux douces of l'Université de Montréal.

production in lakes, Downing et al. (1990) followed the suggestion of Oglesby (1977) in establishing fish production models from rigorously defined and repeatable measurements of biological fish production rather than rough indices of catch. They found, in a study of 23 fish communities in 20 lakes covering a wide range of geographical areas and trophic status, that the production of entire fish communities was closely correlated with annual phytoplankton production, mean total phosphorus concentration, and annual average fish standing stock.

Although Downing et al.'s (1990) analysis makes predictions about the rate of energy transfer between trophic levels in lakes of differing trophic status, most ecologists and fisheries managers are more interested in the production of specific populations of central trophic, economic, or recreational importance. Some analyses have been made of the biological production of other aquatic faunae, most notably zooplanktonic and benthic invertebrates in lakes (Plante and Downing 1989) and streams (Morin and Bourassa 1992), but no broad comparative study has been made of the production of fish populations. The most complete analyses of fish population production to date have been those of Banse and Mosher (1980) and Dickie et al. (1987) that relate the production to biomass ratio (P/B) of fish populations to body-mass. Neither of these analyses examines the influence of ecosystem trophic status or health on fish production, and both are based on small data bases, comparing only 11 and 8 populations, respectively.

Many factors have been hypothesized to influence the productivity of aquatic populations (reviewed by Morgan et al. 1980; Downing 1984), but quantitative tests of real relationships between field fish production estimates and population and environmental characteristics are lacking. Ecologists have observed, however, a positive relationship between production and population biomass (e.g. Waters 1977). If this relationship holds for fish populations, then factors affecting fish standing stock should also have an impact on fish production. Rates of growth decrease and longevity increases with body-mass (Peters 1983); therefore, populations of larger fish should be less productive per unit biomass than small ones. The literature contains some empirical support for this expectation, both in fish (Banse and Mosher 1980; Dickie et al. 1987) and in other aquatic populations (Plante and Downing 1989). The positive effect of temperature on fish production can be inferred from its influence on growth rates (e.g. Goldspink 1979; Quiros 1990) and egg production and development time (e.g. Holčík 1970). In addition, factors mediating food availability to fish populations such as lake primary production and trophic status (Mills 1985; Kelso 1988), pH (Rask 1984), alkalinity (Saunders and Power 1970; Kelso 1988), morphometry (Saunders and Power 1970; Kelso 1988), and climate (Efford 1972; Craig 1980; Rask and Arvola 1985) should also influence the productivity of fish populations.

No broad general synthesis of fish population production rates or analysis of the combined influence of fish population characteristics and environmental conditions on fish population production has yet been published. So far, the multivariate relationship between fish population production and eutrophication, lake morphometry, geography, and climate has not been examined. Such knowledge would be of theoretical importance in identifying the biological and physical characteristics most closely correlated with production, and of practical importance in improving knowledge about factors influencing the rates of renewal of important freshwater fish populations.

This research draws together existing measurements of fish population production to find how the annual production of fish populations is related to their population biomass and body-mass. We also test several hypotheses regarding the influence of lake trophic status and primary production, physical characteristics of the environment, water chemistry, and lake morphometry on rates of fish population production.

Methods

Data on the annual production and standing biomass of fish populations were gleaned from an exhaustive survey of the primary ecological literature published since 1969. We did not consider data on populations supported by stocking, populations stocked every year, or production rates calculated excluding age classes <3 yr to be sufficiently accurate measurements of natural fish production to be included in the analysis. Production and biomass data are expressed in fresh mass. All estimates were actual measures of biological production (as opposed to catch or yield) and were mostly estimated using cohort, Allen curve, or instantaneous growth methods (Chapman 1971; Rigler and Downing 1984). Conversions from energetic or dry mass units were made using published constants (Cummins and Wuycheck 1971). Body-size estimates of individuals in the populations were collected as the maximum individual body-mass (W, grams fresh mass) and were usually determined as the upper limit of largest length class included in production calculations. For a few populations, W was estimated from data on similar species in other lakes or from Scott and Crossman (1973). Data on biotic and abiotic characteristics of lakes were derived from published works or in some cases were obtained through direct communication with the authors of the production studies. Morphometric characteristics collected were lake area and mean and maximum depth. Lake productivity indicators were phytoplankton productivity, total phosphorus, total nitrogen, and chlorophyll *a* concentrations in the water column, and conductivity (as a measure of total ion concentration). Chlorophyll, total nitrogen, and total phosphorus concentrations were collected as annual averages. The concentration of total dissolved solids of several lakes were estimated from conductivity measurements (Schlesinger and McCombie 1983) in order to permit the calculation of the morphoedaphic index (Ryder 1965). Annual mean air temperature, which is closely correlated with surface water temperature (Straškraba 1980), was used instead of water temperature (see also Schlesinger and Regier 1982) because of its greater availability in climatic reports (Wernstedt 1972). Exploitation of populations was simply recorded as presence or absence of fishing activity in lakes. The trophic level of fish populations (i.e. planktivore, benthivore) was determined using information presented in data sources wherever possible or from the diet of similar species in other lakes. Latitude and altitude were also noted where available.

Analyses of relationships between fish population production, population biomass, and body-mass were performed using simple and multiple regression (Gujarati 1978; Draper and Smith 1981) after logarithmic transformation, which stabilized the variance, linearized the responses, and normalized the residuals. The validity of each regression analysis reported below was verified by extensive residual analysis (Draper and Smith 1981). Because data on lake characteristics were rarer than data on fish standing stock and body-mass, analyses of the influence of trophic, physical, and chemical characteristics of lakes were performed by analyzing the residuals of multiple regression equations (Downing et al. 1990), following the standard protocol of Draper and Smith (1981). Analysis of var-

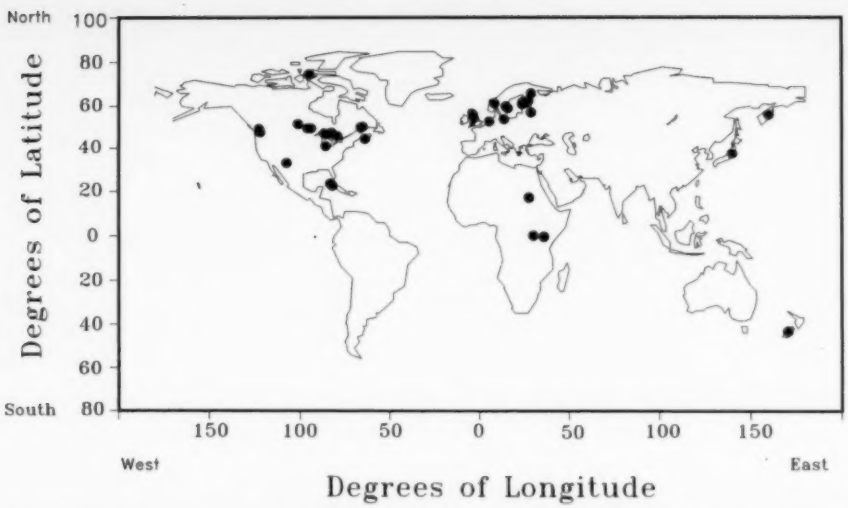

FIG. 1. Location of 38 lakes for which fish population production data were obtained from the published literature. Only 32 different points can be distinguished because many of the Scandinavian lakes are closely spaced.

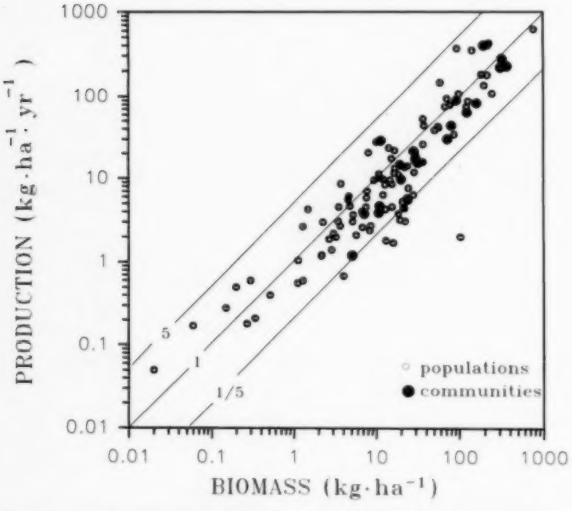

FIG. 2. Relationship between the annual production and annual mean standing biomass of fish populations (data from this study) and communities (data from Downing et al. 1990). The lines represent *P/B* ratios of 0.2, 1, and 5.

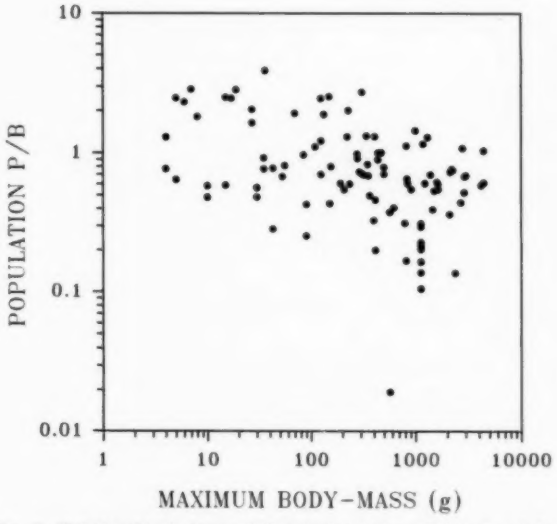

FIG. 3. Relationship between *P/B* calculated from Table 1 and the body-mass of the largest size class in the populations. The outlying point is the arctic Char Lake.

iance of the residuals of multiple regression analyses was used to test for the effect of trophic level and exploitation.

Results and Discussion

Fish production data were obtained for 100 populations (Table 1) from 38 lakes and reservoirs (Table 2) in a wide range of geographic locations (Fig. 1) representing oligotrophic to hypereutrophic lakes in tropical to temperate climates (Table 2). This is nearly 10 times greater than the number of production estimates used in previous studies of fish population production (cf. Banse and Mosher 1980). Fish species spanned a wide range of sizes and trophic levels (Table 1). Populations varied in mean annual standing stock from 0.02 kg·ha^{-1} for *Micralestes acutidens* in the tropical Lake Kariba (Mahon and Balon 1977) to 771 kg·ha^{-1} for *Lepomis macrochirus* in the small, eutrophic, temperate Wyland Lake (Gerking 1962; Mahon 1976).

Biomass and Body-mass

The production of fish populations (P, kilograms per hectare per year) was most strongly correlated with the standing biomass (B, kilograms per hectare) and the body-mass of the largest size class of fish in the population (W, grams):

$$(1)\quad \log_{10}P = 0.32 + 0.94 \log_{10}B - 0.17 \log_{10}W$$

TABLE 1. Production (P, g·ha^{-1}·yr^{-1}), biomass (B, kg·ha^{-1}), and maximum individual mass (W, g) of fish population data drawn from the literature. GR indicates the trophic group (1 = planktivore, 2 = benthivore, 3 = piscivore, 4 = planktivore and benthivore). GR or W values in italics indicate that trophic status or body-mass data were inferred from populations in other lakes. Values of P, B, and W are rounded. Data sources are listed in Table 2.

Lake	Fish species	GR	P	B	W
226 NE	*Coregonus clupeaformis*, 1973	1	4.6	15.7	1127
	Coregonus clupeaformis, 1974	1	6.4	28.3	1127
	Coregonus clupeaformis, 1975	1	7.7	24.8	1127
	Coregonus clupeaformis, 1976	1	4.6	21.5	1127
226 SW	*Coregonus clupeaformis*, 1973	1	3.8	18.8	1127
	Coregonus clupeaformis, 1974	1	3.1	22.6	1127
	Coregonus clupeaformis, 1975	1	3.2	19.5	1127
	Coregonus clupeaformis, 1976	1	1.7	16.2	1127
Alinen Mustajärvi	*Coregonus muksun*	1	11.8	29.1	620
	Perca fluviatilis	2	15.9	37.0	90
	Esox lucius	3	2.6	6.9	571
Big Indian	*Salvelinus fontinalis*	*3*	1.9	2.7	320
Big Turkey	*Salvelinus fontinalis*	2	0.6	1.1	367
	Catostomus commersoni	2	0.7	4.1	817
Bill	*Salvelinus fontinalis*	*3*	0.2	0.3	*1600*
Botjärn	*Perca fluviatilis*	3	16.6	29.4	30
Char	*Salvelinus alpinus*	1	2.0	104.0	*570*
Dalnee	*Gasterosteus aculeatus*	1	22.0	17.0	*4*
	Oncorhynchus nerka	1	350.0	143.0	*125*
	Salvelinus malma	3	26.0	37.0	500
Demenets	*Carassius carassius*	*4*	4.6	7.6	841
	Tinca tinca	*2*	2.0	3.3	1230
	Perca fluviatilis	*3*	78.2	78.0	475
	Rutilus rutilus	*2*	107.9	248.0	152
	Acerina cernua	*2*	23.5	14.4	27
	Esox lucius	*3*	3.7	5.3	1386
	Scardinius erythrophtalmus	*2*	8.6	15.7	208
Elephant Butte	*Ichtiobus bubalus*, 1966	*4*	38.5	51.1	2252
	Carpiodes carpio, 1967	—	2.7	3.7	2146
	Cyprinus carpio, 1968	*2*	6.1	4.8	1310
George	*Tilapia nilotica*	1	53.3	37.0	*1000*
Haukilampi	*Perca fluviatilis*	*3*	5.9	7.7	35
Horkkajärvi	*Coregonus pilea*	1	10.2	11.2	280
	Perca fluviatilis	4	5.3	21.0	90
Iso-Mustajärvi	*Perca fluviatilis*	*2*	22.2	28.7	43
Karhujärvi	*Perca fluviatilis*	4	2.4	8.5	43
Kariba	*Schilbe mystus*	*3*	0.2	0.3	53
	Hippopotamyrus discorhynchus	—	424.7	227.1	133
	Heterobranchus longifilis	—	3.1	5.2	4200
	Haplochromis darlingi	—	8.7	3.8	6
	Synodontis nebulosus	—	1.1	1.1	35
	Labeo altivelis	—	4.1	6.8	1630
	Hydrocynus vittatus	—	20.6	30.2	3000
	Clarias gariepinus	—	15.7	35.5	2700
	Tilapia rendalli	*1*	44.0	38.1	1180
	Sarotherodon mossambicus	—	178.5	215.7	350
	Eutropius depressirostris	*3*	4.7	4.9	85
	Alestes lateralis	*3*	366.9	94.8	36
	Mormyrus longirostris	2	6.4	12.3	2900
	Sargochromis codringtoni	—	9.6	9.6	440
	Synodontis zambezensis	—	7.1	7.9	440
	Micralestes acutidens	2	0.1	0.02	5
	Mormyrops deliciosus	3	16.3	30.4	1500
	Malapterurus electricus	—	107.4	99.6	2800
	Marcusenius macrolepidotus	—	4.6	3.5	222
Kiutäjärvi	*Perca fluviatilis*	*3*	11.2	18.7	235
	Rutilus rutilus	*4*	4.3	13.1	400
Konnevesi	*Phoxinus phoxinus*	*4*	9.6	15.0	5
	Nemacheilus barbatulus	—	14.3	124.4	15
Laluisa	*Cichlasoma tetracanthus*	3	8.4	12.9	830
	Lepomis macrochirus	1	181.4	184.0	278
	Micropterus salmoides	3	87.1	128.8	2923
Little Turkey	*Salvelinus fontinalis*	3	1.2	2.2	913
	Catostomus commersoni	2	2.7	8.7	794
Loch Leven	*Salmo trutta*	*3*	17.5	15.6	820
	Perca fluviatilis	3	27.9	10.2	310

TABLE 1. (*Concluded*)

Lake	Fish species	GR	*P*	*B*	*W*
Marion	*Salmo gairdneri*	3	9.8	12.3	*156*
	Oncorhynchus nerka	1	11.6	16.6	*125*
Matamek	*Salvelinus fontinalis*	*3*	2.2	3.1	309
Nakuru	*Sarotherodon alcalicum*	1	38.6	15.7	17
Øvre Heimdalsvatn	*Salmo trutta*	4	13.3	16.9	500
	Phoxinus phoxinus	4	1.4	2.9	10
Red Deer	*Semotilus atromaculatus*	1	0.5	0.2	15
	Pimephalus promelas	4	4.3	1.5	7
	Salvelinus fontinalis	2	0.6	0.3	229
	Notemigonus crysoleucas	1	0.2	0.1	19
	Perca flavescens	2	20.7	8.2	150
	Semotilus margarita	1	2.7	1.3	27
Sabanilla	*Lepomis macrochirus*	1	135.8	198.3	355
	Cichlasoma tetracanthus	3	41.7	57.2	291
	Micropterus salmoides	3	43.0	56.2	2217
Small Spectacles	*Philypnodon breviceps*	1	397.8	219.8	8
Tatsu-numa	*Moroco steindachneri*	4	18.0	31.0	*10*
Tjeukemeer	*Rutilus rutilus*, 1967–68	4	94.6	71.9	340
Tjeukemeer	*Abramis brama*, 1969	4	34.3	86.9	1450
Vitalampa	*Perca fluviatilis*	1	9.8	20.2	30
Warniak	*Esox lucius*, 1969	3	13.7	22.4	4425
Washington	*Esox lucius*, 1970	3	11.3	10.9	4445
	Perca flavescens	3	0.6	1.3	*415*
	Gasterosteus aculeatus	1	0.4	0.5	4
	Spirinchus staleichthus	1	3.1	3.5	—
	Oncorhynchus nerka	1	5.6	4.6	*125*
	Cottus asper	3	74.9	67.9	110
	Mylocheilus caurinus	2	3.0	2.3	408
	Ptychocheilus oregonensis	3	1.8	13.2	2385
West Blue	*Stizostedion vitreum*	3	2.1	5.8	2110
Windermere	*Perca fluviatilis*	*3*	74.6	123.2	192
Wishart	*Salvelinus fontinalis*	3	1.2	2.2	1641
	Catostomus commersoni	2	4.4	22.2	414
Wyland	*Lepomis macrochirus*	4	625.0	771.4	56

($R^2 = 0.84$, $n = 100$, RMS = 0.107, $p < 0.0001$). Analysis of variance of the residuals of this equation showed that there was no significant difference in the production of populations of different trophic levels ($p > 0.05$). The regression coefficients for the effects of biomass and body-mass on production are very similar to those found for aquatic invertebrates (Plante and Downing 1989; 0.79 and −0.16, respectively). Fish populations tend to increase their production slightly more rapidly with increased biomass than do zooplankton and benthos, but the negative influence of body-mass on mass-specific production is virtually identical. The fact that the slope associated with $\log_{10}B$ is close to 1 in equation 1 suggests that P/B does not vary significantly with B. This is similar to the finding of Downing et al. (1990) for fish communities and is important to note, since several authors have suggested that annual secondary production of populations can be estimated employing an average production to biomass ratio (reviewed by Rigler and Downing 1984). Such a procedure would be invalid if P/B were to vary systematically with B, as is the case for freshwater invertebrates (Plante and Downing 1989). P/B in fish populations generally varied between 5 and 0.2 and was not systematically related to the population biomass (Fig. 2). The fact that the slope associated with $\log_{10}B$ is not significantly different from 1 (Fig. 2) suggests that mass-specific renewal rates are not strongly density dependent in freshwater fish populations and that P/B values should translate well from one population of a given species to another.

Fish production was strongly correlated with body-mass. Equation 1 shows that P of fish populations declined as $W^{-0.17}$ where B is entered as a covariate. This shows that P/B declined approximately as $W^{-0.17}$ (Fig. 3). Allometric theory, on the other hand, argues that P/B should decline as $W^{-0.25}$ (Humphreys 1981; Lavigne 1982; Peters 1983; Calder 1984). This expectation was upheld for 11 fish populations by Banse and Mosher (1980). The exponent we obtained by least squares regression in equation 1 is significantly different from −0.25 ($p < 0.05$), but major axis regression (Jolicoeur 1990) shows a functional relationship between P/B and W with an exponent of −0.22. The P/B of a population with W of 5 g would be more than three times, on average, that of a population of 5-kg fish with the same standing biomass (equation 1).

Other Factors Correlated with Freshwater Fish Production

The effect of environmental characteristics was evaluated by examining their correlations with the residuals of equation 1 (Table 3). This procedure is equivalent to the next step beyond equation 1 in a stepwise multiple regression analysis with forward selection (Hocking 1976).

Climatic Variation

Because fish are poikilotherms, temperature should influence rates of energy acquisition and growth. Table 3 shows that fish production was significantly correlated with annual mean air temperature ($p < 0.001$) and latitude ($p < 0.001$). Although highly influenced by the very low rates of production seen in arctic Char Lake (Table 1), the influence of air tem-

TABLE 2. Characteristics of the lakes in which fish production data in Table 1 were estimated. NS = number of species, E = population exploited (yes, no), Alt = altitude of the lake (m), $\bar{z}$ = mean depth (m), Area = ha, T = mean annual air temperature (°C), Cond. = annual average conductivity ($\mu mho \cdot cm^{-1}$), TDS = total dissolved solids ($mg \cdot L^{-1}$), TP = annual average total phosphorus ($\mu g \cdot L^{-1}$), TN = annual average total nitrogen ($\mu g \cdot L^{-1}$), Chlo = annual average chlorophyll *a* ($\mu g \cdot L^{-1}$), PP = primary production ($g\ C \cdot m^{-2}$). Values of TDS in italics were estimated from conductivity measurements (Schlesinger and McCombie 1983). Data sources: 1, D. R. Alexander (Department of Fisheries and Oceans, Moncton, N.B., pers. comm.); 2, Alexander and Merrill (1976); 3, Alexander et al. (1986); 4, Arvola (1983); 5, Balon (1972); 6, Balon (1974); 7, Beattie et al. (1972); 8, Chadwick (1976); 9, Ciepielewski (1981); 10, Coche (1974); 11, Craig (1980); 12, Dunn et al. (1969); 13, W. T. Edmondson (Department of Zoology, University of Washington, Seattle, WA, pers. comm.); 14, Edmondson and Lehman (1981); 15, Efford (1972); 16, Eggers et al. (1978); 17, Eloranta and Palomäki (1986); 18, Ganf (1974); 19, Gerking (1962); 20, Gerking (1964); 21, Goldspink (1978); 22, Goldspink (1979); 23, Gulin and Rudenko (1973); 24, Gwahaba (1973); 25, Holčík (1970); 26, IBP data set; 27, Jester (1971); 28, Jester (1972); 29, Jester (1976); 30, Johansson (1983); 31, J. R. M. Kelso (Great Lakes Fisheries Research Branch, Sault Ste. Marie, Ont., pers. comm.); 32, Kelso (1985); 33, Kelso and Ward (1972); 34, Kelso and Ward (1977); 35, Kloster (1978); 36, Krogius et al. (1972); 37, Lam et al. (1986); 38, LeCren (1976); 39, Lien (1978); 40, Lien (1981); 41, E. A. Lind (Department of Zoology, University of Oulu, Oulu, Finland, pers. comm.); 42, Lind et al. (1973); 43, Lind et al. (1974); 44, Mahon (1976); 45, Mahon and Balon (1977); 46, C. A. Mills (Department of Fisheries and Oceans, Freshwater Institute, Winnipeg, Man., pers. comm.); 47, Mills (1985); 48, Mills and Eloranta (1985); 49, Miura and Tanaka (1975); 50, Morgan (1972); 51, Mortimer and Worthington (1942); 52, Nakanishi (1975); 53, Nyberg (1979); 54, O'Connor and Power (1973); 55, G. Power (Department of Biology, University of Waterloo, Waterloo, Ont., pers. comm.); 56, Ramberg (1976); 57, M. Rask (Lammi Biological Station, University of Helsinki, Helsinki, Finland, pers. comm.); 58, Rask (1984); 59, Rask and Arvola (1985); 60, Rask et al. (1986); 61, Rigler (1972); 62, Rigler (1975); 63, Rigler (1978); 64, Särrkä (1972); 65, Saunders and Power (1970); 66, Scheffer and Robinson (1939); 67, D. J. Staples (Department of Zoology, University of Canterbury, Canterbury, New Zealand, pers. comm.); 68, Staples (1975a); 69, Staples (1975b); 70, Tangen and Brettum (1978); 71, J. E. Thorpe (Freshwater Fisheries Laboratory, Pitlochry, Scotland, pers. comm.); 72, Thorpe (1974); 73, Tikka and Paasivirta (1979); 74, Vareschi (1982); 75, Vareschi and Jacobs (1984); 76, Vareschi and Jacobs (1985); 77, Wali et al. (1972); 78, Ward and Robinson (1974); 79, Zachweja (1973).

Lake	NS	E	Alt	$\bar{z}$	Area	T	pH	Cond	TDS	TP	TN	Chlo	PP	Data source(s)
226 NE 1973	5	No	414	6	8	2	6.5	21	*13*	5	270	11	54	46, 47
226 NE 1974	5	No	414	6	8	2	6.4	23	*15*	5	391	19	74	46, 47
226 NE 1975	5	No	414	6	8	2	6.4	24	*16*	6	470	16	56	46, 47
226 NE 1976	5	No	414	6	8	2	6.5	26	*17*	6	510	10	76	46, 47
226 SW 1973	5	No	414	6	8	2	6.6	20	*13*	4	241	5	44	46, 47
226 SW 1974	5	No	414	6	8	2	6.5	23	*15*	4	352	3	32	46, 47
226 SW 1975	5	No	414	6	8	2	6.5	24	*16*	4	429	8	32	46, 47
226 SW 1976	5	No	414	6	8	2	6.6	27	*17*	3	469	6	28	46, 47
Alinen Mustajärvi	3	—	—	3	1	3	5.8	24	*16*	39	1545	10	7	4, 59
Big Indian	5	No	—	3	106	6	5.5	42	*27*	—	—	—	—	1, 2, 3
Big Turkey	2	—	372	12	52	5	—	41	*27*	4	—	—	2	31, 32, 37
Bill	5	—	96	—	154	1	5.8	23	*15*	—	—	—	—	54
Botjärn	2	—	280	3	10	5	5.6	25	*16*	14	400	—	3	30, 53, 56
Char	1	—	30	10	53	−9	—	196	*128*	4	—	—	4	61, 62, 63
Dalnee	3	—	—	32	136	2	—	—	—	92	—	—	487	36
Demenets	7	—	—	3	7	5	—	—	—	—	—	—	—	23
Elephant Butte	26	No	1646	6	8100	16	8.3	700	303	800	—	—	—	27, 28, 29
George	32	Yes	914	3	27000	22	9.0	220	264	—	2600	100	367	6, 10, 12, 18, 24
Haukilampi	3	—	—	—	2	3	6.8	43	*28*	23	580	—	—	73
Horkkajärvi	2	—	—	7	1	3	5.7	43	*28*	115	973	10	6	4, 59
Iso-Mustajärvi	3	—	—	—	3	3	6.9	62	41	20	290	—	—	73
Karhujärvi	1	—	158	5	1	3	4.4	43	—	148	582	26	16	57, 58, 60
Kariba	28	Yes	485	29	5364	25	8.0	73	58	—	—	—	—	5, 6, 10
Kiutajärvi	5	Yes	225	15	45	0	7.1	51	43	9	350	1	—	41, 42, 43
Konnevesi	2	Yes	95	10	18800	3	7.5	44	*29*	6	420	2	13	17, 48, 64
Laluisa	7	—	—	1	1	25	—	—	—	—	—	—	—	25
Little Turkey	2	—	375	6	19	5	—	39	*25*	6	—	—	1	31, 32, 37
Loch Leven	7	Yes	107	4	1330	9	8.3	—	—	95	8250	100	354	38, 50, 71, 72
Marion	2	No	300	2	13	9	—	18	*12*	246	—	—	8	15, 77
Matamek	4	No	122	—	1620	1	5.9	14	*9*	—	—	—	—	55, 65
Nakuru	1	Yes	1759	2	4400	18	10.5	18167	*11862*	9850	17000	1160	881	74, 75, 76
Øvre Heimdalsvatn	2	—	1090	5	78	2	6.9	15	*10*	15	20	—	12	35, 39, 40, 70
Red Deer	6	—	400	4	6	4	6.9	35	*23*	—	—	—	—	8
Sabanilla	7	Yes	—	1	3	25	—	—	—	—	—	—	—	25
Small Spectacles	3	Yes	610	3	1	9	8.6	119	*78*	140	—	—	—	67, 68, 69
Tatsu-numa	2	Yes	790	4	1	12	6.2	—	—	10	400	0.4	8	51, 49, 52
Tjeukemeer 1967–68	7	Yes	−1	2	2130	10	8.0	—	—	263	1700	30	300	7, 21, 22
Tjeukemeer 1969	7	Yes	−1	2	2130	10	8.0	—	—	263	1700	30	300	7, 21, 22
Vitalampa	1	No	280	3	3	5	5.7	18	*12*	17	425	—	5	30, 53, 56
Warniak 1969	>1	Yes	—	2	38	7	7.8	275	*180*	—	—	—	130	9, 26, 79
Warniak 1970	>1	Yes	—	2	38	7	8.0	226	*148*	—	—	—	—	9, 26, 79
Washington	7	Yes	4	33	8760	11	7.7	81	*53*	35	273	15	157	13, 14, 16, 66
West Blue	5	No	670	11	160	2	8.3	160	*104*	—	—	0.3	38	33, 34, 78
Windermere	>1	Yes	39	22	1482	10	—	—	—	—	—	—	—	11, 38, 51
Wishart	2	—	388	2	19	5	—	33	22	5	—	—	0.7	31, 32, 37
Wyland	5	No	—	—	3	10	8.5	—	—	—	—	—	—	19, 20, 44

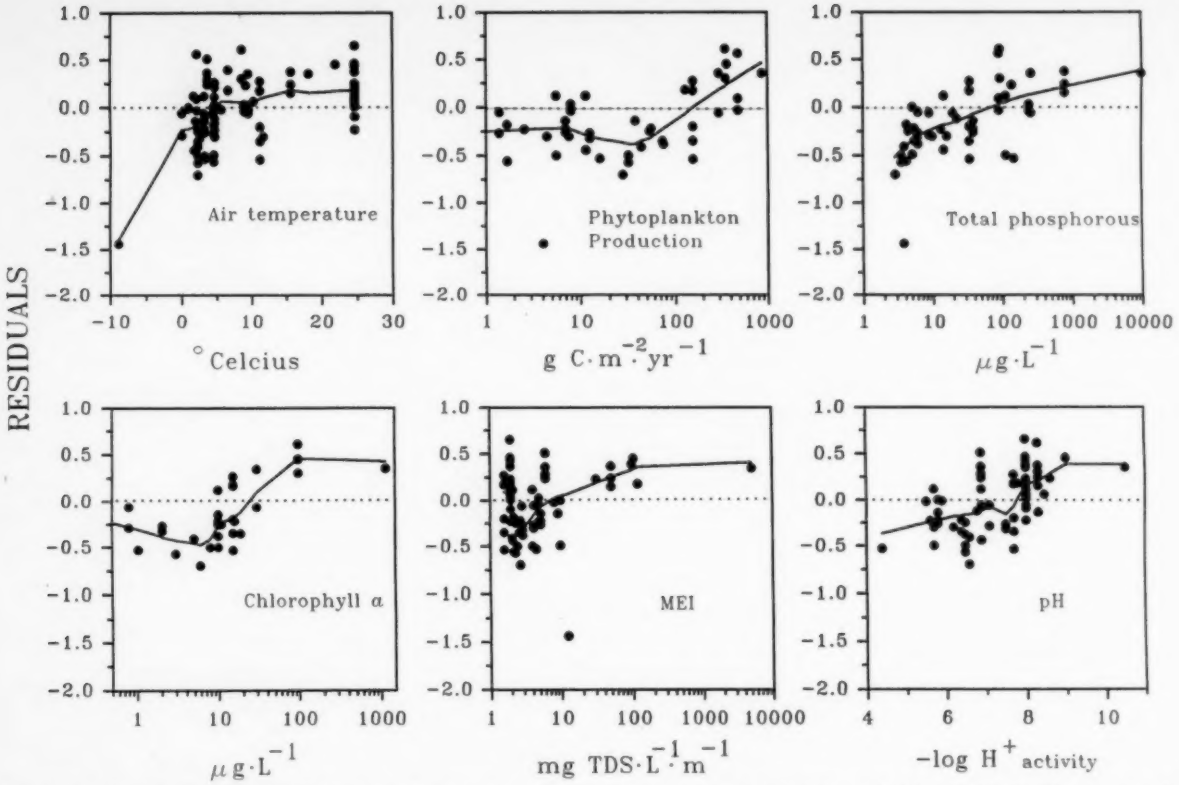

FIG. 4. Relationships of the residuals from equation 1 in $\log_{10}$ form to various characteristics of the study lakes (Table 2). Lines are trends calculated using locally weighted robust sequential smoothing (Cleveland 1979). The outlier in the first three and the fifth panels is the arctic Char Lake.

perature (T, degrees Celsius) on fish production (Fig. 4) can be summarized by the multiple regression equation

$$(2)\quad \log_{10}P = 0.20 + 0.93\log_{10}B - 0.19\log_{10}W + 0.02T$$

($R^2 = 0.88$, $n = 100$, RMS = 0.084, $p = 0.0001$). Analysis of this equation suggests that the ratio of fish production to biomass varies from about 0.9 in temperate oligotrophic Lake 226, on average, to over 2.3 in tropical Lake George for a population of the same body-mass ($W = 5$ g) and biomass ($B = 100$ kg·ha^{-1}). For example, P/B for *Salvelinus alpinus* found in Char Lake ($T = -9$) was only 0.02 (Table 1). Figure 4 suggests that, on average, populations found where the average annual air temperature is 20°C have production rates three times those found in similar populations at 0°C.

Lake Trophic Status and Productivity

The ultimate limit to the rate of production of a population of fish must be set by the primary productivity of the ecosystem in which it lives. Downing et al. (1990) found that the productivity of entire fish communities was well correlated with both algal production and the total phosphorus concentration of the water column, an indicator of lake trophic status. Correlation analyses of the residuals of equation 1 show that fish population production is significantly ($p < 0.001$) correlated with total phosphorus concentration, chlorophyll *a* concentration, and algal primary productivity. Figure 4 suggests that, if these correlations reflect underlying trophic mechanisms, a change in total phosphorus concentration from 10 to 100 μg·L^{-1} should be correlated with a threefold increase in P/B of fish populations. Including total phosphorus concentration (TP, micrograms per litre) in a multiple regression analysis with population biomass and body-mass yields

$$(3)\quad \log_{10}P = -0.25 + 0.90\log_{10}B - 0.15\log_{10}W + 0.29\log_{10}\mathrm{TP}$$

($R^2 = 0.81$, RMS = 0.083, $n = 52$, $p < 0.0001$). Lakes with low phytoplankton biomass and production also have lower rates of mass-specific fish production than lakes with phytoplankton biomass >20 μg·L^{-1} and phytoplankton production >200 g C·m^{-2}·yr^{-1} (Fig. 4). Other factors related to lake trophic status, such as total nitrogen concentration and total dissolved solids, were also significantly correlated with fish productivity (Table 3). The morphoedaphic index (Fig. 4), a staple predictor of fishing yields from exploited fisheries (Ryder 1982; Leach et al. 1987), was not significantly ($p > 0.05$) correlated with fish production when biomass and body-mass effects were considered (Table 3). The analysis of fish production presented here upholds Downing et al.'s (1990) contention that fish production is more closely correlated with primary production, phosphorus concentration, and fish standing stock than with the morphoedaphic index.

Recent research has attempted to determine the relative importance of producers and consumers in the control of the structure of communities. The positive relationship between fish

TABLE 3. Correlations between residuals from equation 1 (observed minus predicted P) and lake characteristics. Units are in Table 2. p is the probability level associated with the hypothesis that the lake characteristics and the residuals are uncorrelated. Logarithm transformations were performed where necessary to linearize responses and normalize the residuals. Values of p marked with an asterisk are significant at $p \leq 0.05$ after sequential Bonferroni correction for multiple comparisons (Rice 1989).

Lake characteristic	Pearson coefficient	Number of populations	Number of lakes	p
Total phosphorus (log)	0.62	52	26	<0.001*
Primary production	0.58	47	23	<0.001*
Chlorophyll (log)	0.56	32	14	<0.001*
pH	0.56	74	28	<0.001*
Primary production (log)	0.49	47	23	<0.001*
Species richness (log)	0.49	97	35	<0.001*
Air temperature	0.47	100	38	<0.001*
Latitude	−0.45	100	38	<0.001*
Total dissolved solids (log)	0.36	77	29	0.002*
Total nitrogen (log)	0.46	37	18	0.004*
Conductivity (log)	0.33	77	29	0.004
Lake area (log)	0.27	99	37	0.008
Altitude	0.25	73	27	0.03
Morphoedaphic index (log)	0.21	72	28	0.07
Mean depth (log)	0.00	94	23	0.99

population production and phosphorus concentration suggests a "bottom-up" control of these ecosystems, that is, that each trophic level is limited by the subordinate level. This agrees with McQueen et al.'s (1986) model which predicts that the maximum potential biomass of each trophic level in freshwater pelagic ecosystems is controlled by nutrient availability. This has also been hypothesized for terrestrial ecosystems (see White 1978).

Lake Acidification

Several authors have how discussed the negative impact of lake acidification on fish growth and productivity (e.g. Beamish et al. 1975; Haines 1981; Rask 1984; Schindler et al. 1985; Ingersoll et al. 1990). Whole-lake experiments (Schindler et al. 1985) have shown that fish production falls dramatically with decreases in pH and fish species are nearly completely eliminated in lakes that fall to pH 4 (Haines 1981; Rahel and Magnuson 1983). Analysis of the residuals of equation 1 extend general, worldwide support to the results of manipulations and individual case studies because mass-specific fish production rates rise by nearly an order of magnitude between acid (pH = 4) and alkaline (pH = 9) lakes (Fig. 4). This result agrees with Brylinsky's (1980) work on pH and phytoplankton production and upholds the negative effect of low pH on benthic invertebrate biomass suggested by the work of Roff and Kwiatkowski (1977) and the negative effect of low pH on invertebrate production seen by Plante and Downing (1989). Low production rates of fish populations in acidic lakes may be the result of low food production. For example, Mills et al. (1987) found that certain species of fish in acidified lakes appeared to starve to death even though the pH was within physiologically tolerable limits.

Species Richness

The species richness of fish communities surveyed ranged from 1 to 32. We found that fish populations in lakes with greater numbers of fish species had higher rates of production than lakes with more depauperate faunae (Table 3). This result probably arises from the tendency for eutrophic lakes to have greater species richness as well as greater mass-specific production rates than oligotrophic lakes (Table 2). Comparison of Downing et al.'s (1990) data on fish community production with our population production data (Table 1) suggests that fish community production is marginally lower (ANCOVA, $p = 0.06$) than fish population production for a given biomass (Fig. 2). Intraspecific competition may therefore lower the rate of production of a given biomass of fish if this fish biomass is partitioned into many species.

Renewal and Exploitation of Fish Populations

An unexploited fish population has a rate of production that, when taken over the long term, balances rates of natural mortality. If this were not the case, then fish populations would either increase toward infinite biomass or fall to extinction. The idea behind scientific fisheries management is that we attempt to replace a portion of the natural mortality with fishing mortality and we hope that removal will be concentrated on the least productive part of the population leading to increased production in the residual population. Fishing yield can therefore never be maintained at a level higher than the production of the population without leading to a decline in the biomass of the exploited populations. Even exploitation near the production rate may lead to recruitment failure. The rate of production examined here therefore sets the extreme upper limit of the rate at which fish populations can be exploited. The real upper limit is closer to the difference between fish production and the irreducible rate of natural mortality found in the population exploited at the maximum sustainable level, as long as adequate recruitment can be maintained.

Simultaneous measures of fish production and sustainable yield are very rare. Downing et al. (1990) found that primary production was a good predictor of fish community production and compared measured production levels with sustained fishing yields found in other lakes of known primary productivity (Oglesby 1977). Although their analyses of this small data base are speculative, they suggest that about 10% of the community fish production can be fished on a sustainable basis although data from extremely large lakes and tropical pisciculture ponds suggest that rates of irreducible natural mortality are proportionately greater in oligotrophic lakes than in eutrophic ones. Downing et al.'s (1990) analysis showed that biological fish production and rates of sustainable yield are strongly corre-

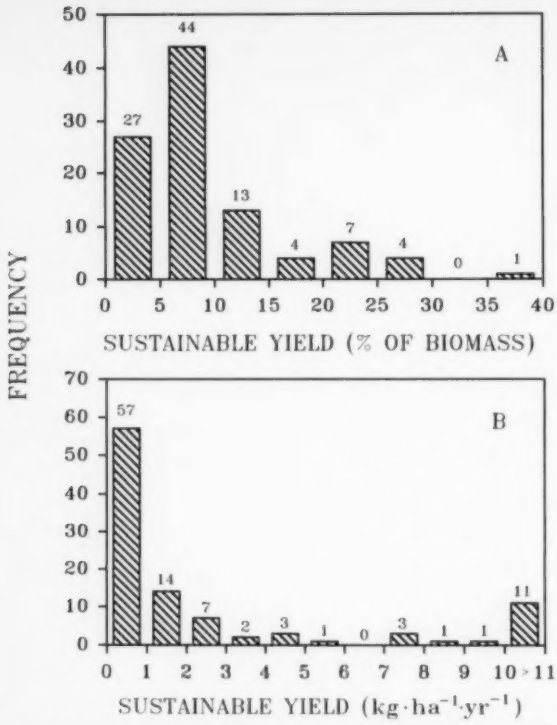

FIG. 5. Frequency histograms of annual sustainable yield (A) as a fraction of biomass, assuming that 10% of the fish production can be removed on a sustainable basis, and (B) in $kg \cdot ha^{-1} \cdot yr^{-1}$, assuming that 10% of the fish production can be harvested on a sustainable basis.

lated; therefore, factors influencing rates of fish population production should also influence sustainable yields.

Assuming that sustainable yields are about 10% of biological production, our data (Table 1) suggest that only a small fraction of the standing fish biomass can be removed on a sustainable basis. Although such calculated sustainable yields go as high as 50% of standing biomass in populations of small, tropical fish, larger fish under arctic conditions (e.g. *Salvelinus alpinus* in Char Lake) can apparently yield as little as 0.2% of standing biomass on an annual basis (Fig. 5). More than one quarter of the fish production data we collected indicated sustainable yields of <5% of biomass, while >85% were <15% (Fig. 5). The data suggest that sustainable yields can be as little as 0.01 $kg \cdot ha^{-1} \cdot yr^{-1}$ or as great as 62 $kg \cdot ha^{-1} \cdot yr^{-1}$ but the majority of sustainable fish populations yields in lakes are <1 $kg \cdot ha^{-1} \cdot yr^{-1}$, while more than 80% are <4 $kg \cdot ha^{-1} \cdot yr^{-1}$. Our calculations are highly speculative due to the lack of data on maximum sustainable fish yield concurrent with production measurements. Equations 1, 2, and 3 and Table 3 show, however, that sustainable fish yields should be lower for larger fish species, at high latitudes, or under acid conditions, and fish yields should be higher at high temperatures or under more eutrophic conditions.

Exploited populations appear to have higher rates of production than unexploited populations of the same biomass and body-size. ANOVA of the residuals of equation 1 shows that the mean residual (in $\log_{10}$ form) of fish populations in unexploited lakes was -0.124, while the mean residual in exploited lakes was 0.118 ($p = 0.03$). This average difference of 0.24 unit suggests that production rates in exploited lakes are about 70% higher (i.e. $10^{0.24} = 1.74$) than fish population production in unexploited lakes. This is a conservative analysis, since rates of exploitation were not measured but only entered as presence or absence of exploitation, but it agrees well with the general belief that exploited populations are composed of younger, faster growing fish.

Acknowledgments

This research was supported by an operating grant to J. A. Downing from the Natural Sciences and Engineering Research Council of Canada (NSERC), a team grant from the Ministry of Education of the Province of Québec, a NSERC fellowship to Mario Henri who performed some of the initial search for data, a CAFIR grant from the Université de Montréal, and the support of OMC Canada. We are grateful to Sophie Lalonde for help in data compilation and analysis and to Ray Oglesby and an anonymous reviewer for suggestions on the manuscript. We would like to especially thank D. R. Alexander, W. T. Edmondson, J. R. M. Kelso, E. A. Lind, C. A. Mills, G. Power, M. Rask, D. J. Staples, and J. E. Thorpe, who generously supplied us with unpublished data.

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pH Tolerance and Metal Toxicity in Populations of the Planktonic Diatom *Asterionella*: Influences of Synthetic and Natural Dissolved Organic Carbon

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Two clones of *Asterionella ralfsii* cf *americana* Körn. were more tolerant of acidic pH with respect to batch culture growth rate than their congener, *A. formosa* Hass. The relative inability of *A. formosa* to satisfy trace metal requirements at lower pH appeared to be a major factor in its reduced tolerance of acidic conditions. The pH tolerance of *A. formosa*, and at times *A. ralfsii*, also could be modified when grown in media containing different concentrations of artificial or natural forms of dissolved organic carbon (DOC). Mixed trace metal additions (Cu, Zn, Mn, Fe, and Al) further affected pH tolerance in *A. ralfsii* both by alleviating limitation and by inducing toxicity, depending on the nature and concentration of DOC. Overall, our results were consistent with pH classification schemes for both *A. ralfsii* and *A. formosa* as they are commonly used in paleolimnological pH reconstruction models. However, apparent pH classifications for both *Asterionella* species could be altered by changes in DOC-mediated trace metal bioavailability. Their assignment to a specific pH category or distribution thus may not represent an inherent biological property of that taxon, but may strongly depend on chemical conditions which often vary in humic lakes subject to acid deposition.

Quant au taux de croissance en culture discontinue, deux clones d'*Asterionella ralfsii* cf *americana* Körn. toléraient mieux le pH acide que leur congénère *A. formosa* Hass. La relative inaptitude d'*A. formosa* à se conformer aux exigences relatives aux métaux à l'état de traces à bas pH semble constituer une cause majeure de sa tolérance réduite á des conditions acides. La tolérance au pH d'*A. formosa* et parfois d'*A. ralfsii* peut aussi être modifiée par leur culture dans des milieux contenant des formes naturelles ou artificielles, à différentes concentrations, de carbone organique dissous (COD). Des additions de mélanges de métaux à l'état de trace (Cu, Zn, Mn, Fe et Al) abaissaient davantage la tolérance au pH d'*A. ralfsii* à la fois en réduisant les limites et en provoquant une toxicité, selon la nature et la concentration du COD. Au bilan, nos résultats correspondent aux systèmes de classification selon le pH d'*A. ralfsii* et *A. formosa* d'usage courant dans les modèles paléolimnologiques de reconstruction du pH. Cependant, les classifications apparentes selon le pH des deux espèces d'*Asterionella* peuvent être modifiées par suite de changements de la biodisponibilité des métaux à l'état de traces en fonction du COD. La distribution ou l'attribution de ces espèces à une catégorie donnée de pH peut donc ne pas représenter une propriété biologique inhérente de ce taxon, mais peut dépendre fortement des conditions chimiques, d'ailleurs souvent variables dans les lacs humiques qui sont sujets au dépôt acide.

Received February 20, 1992
Accepted July 8, 1992
(JB413)

Reçu le 20 février 1992
Accepté le 8 juillet 1992

Anthropogenic acidification changes the phytoplankton species composition of many clearwater lakes in north temperate ecosystems. However, responses of phytoplankton communities to acidification in lakes that contain high concentrations of dissolved organic carbon (DOC) (hereafter called humic lakes) are less well understood. Several mechanisms may explain the apparent influence of DOC on phytoplankton species composition in acidic lakes. Of four major hypotheses regarding pH-dependent changes in phytoplankton growth and community composition (changes in trace metal bioavailability, nutrient availability, dissolved inorganic carbon (DIC) speciation and concentration, and zooplankton grazing; Stokes 1986), the first two processes are most likely to be influenced by the presence of DOC. Nutrient availability can be directly affected by DOC chemical interactions (e.g. for P; Francko 1986; Jones 1990) or indirectly by complexing inorganic Al that could otherwise complex PO_4, thus affecting P bioavailability (Nalewajko and Paul 1985; Jansson et al. 1986).

DOC-mediated changes in phytoplankton species composition also may be a consequence of the pH-dependent control of trace metal activity and, hence, bioavailability. Trace metals have long been thought to affect phytoplankton growth and species succession in acidified lakes because pH strongly affects metal concentration, chemical speciation, and bioavailability (Campbell and Stokes 1985; Stokes 1986). DOC complexation is well known to ameliorate metal toxicity in phytoplankton (Sunda and Guillard 1976; Morel et al. 1978; Sunda and Lewis 1978; Toledo et al. 1982; Sedlacek et al. 1983; Starodub et al. 1987; Riseng et al. 1991), and metal toxicity is also known to be pH dependent (Peterson et al. 1984; Campbell and Stokes

1985; Peterson and Healey 1985; Gensemer 1991a).

The composition of sedimentary diatom communities is used extensively to infer the historical pH and associated chemical composition of lakes (Davis 1987; Charles et al. 1989; Smol et al. 1991). Diatom species composition also correlates with DOC (Anderson et al. 1986; Walker and Paterson 1986; Huttunen et al. 1988; Taylor et al. 1988; Eloranta 1990), and this relationship has been developed such that past DOC concentrations can be inferred (Davis et al. 1985; Kingston and Birks 1990). However, DOC can in turn affect the accuracy of diatom-inferred (DI) pH reconstructions. Taylor et al. (1988) found that DI pH levels for humic lakes in central Ontario often were lower than measured pH, perhaps owing to the influx of allochthonous peat or bog taxa, or because humic lakes possess a different diatom flora than clearwater lakes at similar pH. DI pH reconstructions for the humic Lake Kejimkujik, Nova Scotia (Duthie 1989), and Little Echo Pond, New York (Charles et al. 1990), were difficult to interpret because both were highly sensitive to the abundance of a single dominant taxon, *Asterionella ralfsii* var. *americana*. *Asterionella ralfsii* var. *americana* Körn. is one of the few planktonic diatoms that remains abundant at pH < 5.5–6 (Charles 1985; Davis 1987; Charles et al. 1990), thus making it a useful indicator species in lake acidification studies (Schindler et al. 1985; Findlay and Kasian 1986). The DI pH profile for Kejimkujik suggested that the lake has always been acidic (pH < 5), yet apparently pH increased 0.2–0.3 unit after 1950 concomitant with a decrease in the relative abundance of *A. ralfsii*. Depending on how the ecological preferences of this dominant taxon are interpreted, its reduced abundance may result from either an increase in pH or a decrease in DOC concentration (Duthie 1989).

Our present study examines growth rates of *A. ralfsii* var. *americana* to describe and compare its physiological tolerance of pH in both humic and clearwater systems. Cultures of *A. ralfsii*, isolated from both a clearwater acidic and a humic acidic lake, were grown in both artificial and natural lake water media of different DOC concentration and type to examine the influence of DOC on its pH tolerance. We also used an isolate of *A. formosa* Hass. to compare responses of *A. ralfsii* with a closely related congener that prefers circumneutral waters (Charles 1985; Huttunen and Turkia 1990; Round et al. 1990). Experimental pH tolerances were then compared with paleolimnological pH classification schemes to independently test their biological significance and accuracy for these taxa in humic lakes. Additionally, the influences of mixed trace metal additions on *A. ralfsii* were examined in both artificial and natural lake water media to assess the importance of trace metals in controlling pH tolerance.

Materials and Methods

Algal Isolation and Maintenance

Three collection sites were chosen to represent lakes of different acidity and total DOC concentration (Table 1). From each of these collection sites, a clone of *Asterionella* was isolated from lake water samples which were enriched with 10 μmol $P \cdot L^{-1}$, 50 μmol $Si \cdot L^{-1}$, and 50 μmol $N \cdot L^{-1}$ (as $NaNO_3$). Lake Kejimkujik was chosen to represent a humic, acidic lake (Table 2) from which an *A. ralfsii* clone could be isolated that most closely corresponds to populations represented in the sediment core from Duthie (1989). *Asterionella ralfsii* also was isolated from Plastic Lake, Ontario, Canada, to study a clone from a relatively clear, acidic lake (Table 2). *Asterionella formosa* was isolated from Lake Huron to examine a congeneric taxon that is widely distributed among clear, circumneutral freshwater habitats. All were isolated from single colonies at least twice to ensure clonal integrity, and all were rendered axenic without antibiotics by repeated washing and reisolation under sterile conditions.

Cultures were isolated and maintained in 50-mL Pyrex tubes (Corning Glass Works, Corning, NY) containing 40 mL of a modified FRAQUIL growth medium (Morel et al. 1975). Medium modifications consisted of increasing Si concentrations to 50 μmol $\cdot L^{-1}$ and adding 200 μmol $B \cdot L^{-1}$ to enhance diatom growth. FRAQUIL was further modified for the *A. ralfsii* clones by lowering the concentration of the synthetic metal chelator EDTA (ethylenediaminetetraacetic acid, disodium salt; BDH Chemicals, Ltd.) to 0.5 μmol $\cdot L^{-1}$ without changing total metal concentrations (Gensemer 1991a) and by the addition of 1 mmol $\cdot L^{-1}$ of the pH buffer MES (2-(*N*-morpholino)ethanesulfonic acid, $pK_a = 6.15$; Sigma Chemical Co.). MES is commonly used in algal cultures and apparently does not complex significant amounts of trace metal ions (Wehr et al. 1986). All maintenance media and glassware were sterilized by autoclaving while partially filled with deionized, distilled water (DDW). Media stock solutions were prepared from reagent-grade chemicals and DDW. Cultures were incubated in a constant-environment chamber (Percival Mfg. Co., Boone, IA) held at 18°C and illuminated with cool-white fluorescent bulbs at a PAR flux of approximately 180 μeinst $\cdot m^{-2} \cdot s^{-1}$.

Experimental Media Preparation

Experiments utilized both artificial and nutrient-enriched natural lake water growth media. The artificial medium FRAQUIL was used as modified above, but Si and B levels were reduced to 25 μmol $Si \cdot L^{-1}$ and 100 μmol $B \cdot L^{-1}$ to limit stationary-phase densities to practical levels. *Asterionella formosa* would not grow reliably at the same EDTA concentration (= 0.5 μmol $\cdot L^{-1}$) as the *A. ralfsii* clones (R. W. Gensemer, unpubl. data); therefore, experiments using *A. formosa* were run at both 0.5 μmol $\cdot L^{-1}$ and at the normal FRAQUIL EDTA compliment of 5 μmol $\cdot L^{-1}$. pH was set in all media prior to sterilization by additions of 1 N NaOH or HCl using a Radiometer GK2401C combination pH electrode (Radiometer, Copenhagen). Media were then sterilized by filtration directly into 28-mL polycarbonate centrifuge tubes (Nalge Co.) using 20-cc syringes and 25-mm-diameter filter housings containing 0.2-μm polycarbonate membranes (Poretics Co.) which were autoclaved before use. To prevent steam contamination during autoclaving, tubes were sterilized by partially filling each with DDW and heating in a 95°C water bath for 2 h (Brand et al. 1983). The tubes were rinsed with DDW which was then discarded.

Growth media were also prepared using samples from Plastic Lake, Ontario, and Lake Kejimkujik, Nova Scotia, to compare pH tolerances in waters of relatively low versus high concentrations of DOC, respectively (Table 2). Additional experiments were run for a single clone (KjAr-20) using water from both the eastern and western portions of Big Dam Lake in Kejimkujik Park, Nova Scotia. Big Dam Lake is separated by a narrows into two basins which exhibit similar major ion composition, yet differ primarily with respect to total DOC (and to a lesser extent, Fe and Al) concentration by approximately threefold (Table 2) (see also Freedman et al. 1989). Lake water samples were collected from a depth of approximately 1–2 m

TABLE 1. Algal isolation information for *Asterionella* cultures, including species identifications, isolation sites and locations, and clone designations (UTCC = University of Toronto Culture Collection).

Species	Isolation site	Location	Clone designation	UTCC No.
Asterionella ralfsii var. *americana* Körn.	Lake Kejimkujik	Annapolis and Queens counties, Nova Scotia (44°23′N, 65°15′W)	KjAr-20	262
Asterionella ralfsii cf *americana* Körn.	Plastic Lake	Dorset, Ontario (45°11′N, 78°50′W)	PL-10	261
Asterionella formosa Hass.	Lake Huron (Colpoy's Bay)	Wiarton, Ontario (44°49′N, 81°03′W)	GB-15	259

TABLE 2. Growth media chemistry for pH tolerance experiments. FRAQUIL data represent nominal values (including modifications as discussed in text) from Morel et al. (1975) unless otherwise indicated. All other water chemistry data are measured values from the Ontario Ministry of the Environment (Plastic Lake) or Environment Canada laboraory in Moncton, N.B. (Lake Kejimkujik and Big Dam Lake). For natural lake water media, FRAQUIL concentrations of P, Si, and N were added to background concentrations shown below and measured as soluble reactive P (SRP) and Si (SRSi).

	FRAQUIL	Plastic Lake	Lake Kejimkujik	Big Dam Lake East	Big Dam Lake West
pH	As set	5.75	5.8	5.8	5.0
DOC ($mg \cdot L^{-1}$)	0.06 or 0.6	2.1	6.1	2.3	8.0
Gran alkalinity ($mg \cdot L^{-1}$)	—	1.7	1.6	0.9	0.1
Total P ($\mu mol \cdot L^{-1}$)	10	—	0.2	0.2	0.3
SRP ($\mu mol \cdot L^{-1}$)	9.5 ± 0.02	9.4 ± 0.02	8.7 ± 0.1	—	—
Reactive Si ($\mu mol \cdot L^{-1}$)	25	<8	71.4	31.1	78.6
SRSi ($\mu mol \cdot L^{-1}$)	25.1 ± 0.1	32.7 ± 0.7	56.0 ± 0.3	—	—
Total N ($\mu mol \cdot L^{-1}$)	100	<0.9	14.3	8.6	15.0
Dissolved SO_4 ($\mu mol \cdot L^{-1}$)	285	68.8	26.0	22.9	19.8
Dissolved Ca ($\mu mol \cdot L^{-1}$)	250	45.5	16.5	16.5	15.2
Dissolved Mg ($\mu mol \cdot L^{-1}$)	150	19.1	16.0	17.3	14.4
Dissolved K ($\mu mol \cdot L^{-1}$)	20	5.0	8.2	5.1	6.4
Dissolved Na ($\mu mol \cdot L^{-1}$)	285	22.2	121.7	126.1	143.5
Dissolved Cl ($\mu mol \cdot L^{-1}$)	501	11.0	109.8	109.8	112.7

into acid-cleaned 20-L polypropylene carboys (Nalge Co.) using a submersible electric pump containing no metal impeller components (Little Giant Pump Co., Oklahoma City, OK). Samples were filtered as soon as possible after collection through a 47-mm-diameter GF/F filter (Whatman International Ltd., Maidstone, England) and then through a pleated 0.2-μm capsule filter (Gelman, Inc., Ann Arbor, MI) using a peristaltic pump. Filtered samples were refrigerated at 2–4°C and stored in the dark until use. Prior to experimentation, lake water samples were brought to ambient temperature; P, Si, N, and MES were added at the same concentrations as in FRAQUIL, and the solutions were sterile filtered into polycarbonate tubes as described above.

pH Tolerance Experiments

These experiments were designed to examine growth rates at pH values ranging from neutral to slightly below those found in Lake Kejimkujik and Plastic Lake. Treatments consisted of 6 pH levels ranging from 4.5 to 7 separated by 0.5 pH unit, with three replicates per treatment. pH was measured at both the start and end of experiments and only deviated over this time an average of 0.14 pH unit (SD = 0.17, $N = 72$). Cultures were preconditioned by growth in 250-mL batches to late logarithmic phase in the appropriate media set to the pH in which each clone was maintained (approximately pH 5.4 for both *A. ralfsii* clones and approximately pH 7.0 for *A. formosa*). About 20 mL of sterile medium was inoculated with less than 1 mL (usually 0.5) of the preconditioned culture, thereby yielding an initial density of approximately 300–500 $cells \cdot mL^{-1}$.

Metal Addition Experiments

We used one of our *A. ralfsii* cultures (KjAr-20) to examine how metal additions affected pH tolerance in all three medium

TABLE 3. Target concentrations ($\mu g \cdot L^{-1}$) for trace metal additions to natural lake water media. Data are based on all available measurements for unamended Lake Kejimkujik water samples. Treatments labelled "2× + TM" or "10× + TM" denote metal additions 2× or 10× higher than measured control concentrations, respectively. Numbers in parentheses represent standard errors ($N = 3$) when possible for lake water samples without metal addition (= control). Numbers for all metal addition treatments represent single estimates based on the mean Kejimkujik lake water control values.

Metal	Lake Kejimkujik control	Plastic Lake control	Treatment	
			2× + TM	10× + TM
Cu	0*	0*	1	5
Zn	40.7 (10.1)	55	81	407
Mn	32.2 (3.9)	45	65	323
Fe	143.3 (8.8)	6	287	1433
Al	116	18	232	1160

*All measurements were below limit of detection; 2× and 10× levels are estimates based on these limits.

TABLE 4. Trace metal concentrations ($\mu g \cdot L^{-1}$) for FRAQUIL metal addition experiments using clone KjAr-20. Control data are based on analytical measurements; Al concentrations in parentheses are detectable trace values below the limit of detection. All other values represent nominal concentrations based on target data from Table 3. Metal addition treatment designations are as described in Table 3.

Metal	Control (0.5 μmol EDTA·L^{-1})	Control (5 μmol EDTA·L^{-1}	Treatment	
			2× + TM	5× + TM
Cu	0.1	0.1	1	2.5
Zn	21	21	81	203
Mn	31	31	65	162
Fe	26	32	286	717
Al	(7)	(7)	232	580

types. For these experiments, a metal mixture was added rather than making presumptions as to which individual metal was the most important biologically. Five of the more abundant metals (Al, Fe, Mn, Zn, and Cu) were added to lake water media at concentrations that most closely resembled that of our Lake Kejimkujik samples (Table 3). Deposition of strong acids can increase concentrations of several metals in lakes up to 2–5× (LaZerte 1986; Mach and Brezonik 1989; Nelson and Campbell 1991) with the exception of Al concentrations which can increase 10× or more when pH falls below 5 (Driscoll and Schecher 1990). Therefore, metal mixtures were added at natural lake water ratios to levels 2× and 10× contemporary Lake Kejimkujik concentrations for both Plastic Lake and Lake Kejimkujik media. Metals were also added to FRAQUIL media containing 0.5 and 5.0 μmol EDTA·L^{-1} to compare results at two different concentrations of this synthetic chelator. However, the 5× metal additions were used in lieu of 10× (Table 4) to compensate for the substantially lower total DOC concentrations of these media (Table 2).

For metal addition experiments, media were not filtered after metal additions to avoid removal of insoluble metal hydroxide or metal–DOC complexes. To minimize culture contamination, media containing all added nutrients and buffer were filtered through 0.2-μm membranes prior to metal addition. Metals were added and pH was set using sterile-filtered trace metal, HCl, and NaOH stock solutions under conditions that were as free from particulate contamination as possible.

Growth Rate Determination

Population growth rates were measured in batch culture using an in vivo fluorescence technique similar to that of Brand et al. (1981). Fluorometer readings (Sequoia-Turner model 450, Mountain View, CA) from culture tubes acclimated to subdued room light for 10–15 min were recorded at the same time daily (±1 h) until fluorescence values stopped increasing over time (usually 7–12 d). Instantaneous growth rates (per day) were determined as the slope of the linear portion (for at least 4 d of growth) of the relationship between days and ln-transformed fluorescence units.

To ensure that fluorescence-based growth rates were accurately representing population growth rates, cell counts were compared with fluorescence units in representative treatments. Subsamples (3–4 mL) were removed immediately after fluorescence measurements on at least three separate days during logarithmic-phase growth, as determined from graphical examination. Subsamples were preserved in Lugol's solution, and at least 100 colonies were counted to determine cell and colony numbers per millilitre in a 1-mL Sedgwick-Rafter chamber at 100× magnification. If in vivo fluorescence values increase at the same rate as cell numbers, then the slope between ln-transformed fluorescence values and ln-transformed cell densities should equal 1.0 (Brand et al. 1981). This slope for pH tolerance experiments ($=0.958$, SE $= 0.31$, $N = 96$) was not significantly different from 1.0, and for metal addition experiments the slope ($=0.846$, SE $= 0.037$, $N = 60$) was lower, but slope deviations of about 0.1 are not considered to indicate significant inaccuracy (Brand et al. 1981). Therefore, in vivo fluorescence values provided an accurate representation of population growth rate regardless of pH or degree of metal addition.

Water Chemistry

Total trace metal concentrations were measured for all blank media (Tables 3 and 4) at the University of Waterloo Water Quality Laboratory and the Ontario Ministry of the Environment (Dorset, Ont.) using standard methods of atomic absorption spectroscopy. FRAQUIL Al concentrations were measured using a colorimetric reaction with pyrocatechol violet (PCV, Sigma Chemical Co.) as described by Sullivan et al. (1986) and LaZerte et al. (1988). Total Al was measured after acidification to pH 1 (with 50% v/v HCl) for at least 24 h, with a PCV reaction time of 40 min for samples and standards. Soluble reactive Si and P concentrations were measured using standard colorimetric techniques (Strickland and Parsons 1972) after filtration through 0.4-μm polycarbonate membranes.

Data Analyses

The significance of pH and medium type or pH and trace metal addition treatments on algal growth rates was determined using two-way analysis of variance (AOV) routines (SYSTAT, Inc., Evanston, IL) unless otherwise specified. Nominal pH values were used as pH treatment levels for statistical purposes only. If AOV's were significant, a Tukey's HSD test was used to determine which individual treatments were significantly different from one another. A significance level of $p < 0.05$ was chosen for all analyses.

Results

pH Tolerance Experiments

Growth of the *A. ralfsii* clone isolated from Lake Kejimkujik (KjAr-20) was largely unaffected by variations in pH (Fig. 1).

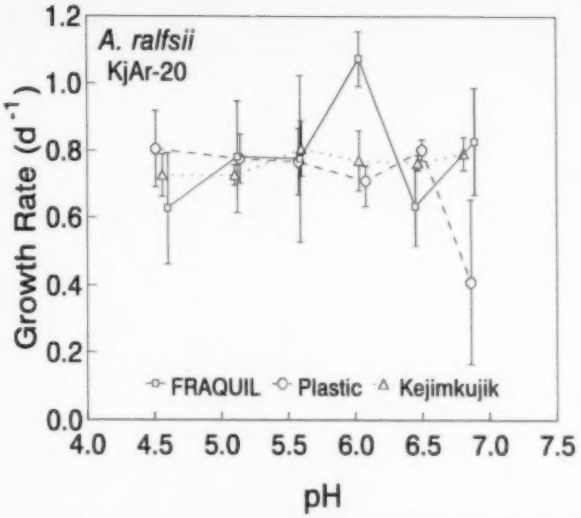

FIG. 1. Instantaneous growth rates as a function of initial experimental pH for the Lake Kejimkujik clone of *A. ralfsii* (KjAr-20) when grown in FRAQUIL, Plastic Lake, and Lake Kejimkujik media. Error bars represent ±2 SE about each mean growth rate.

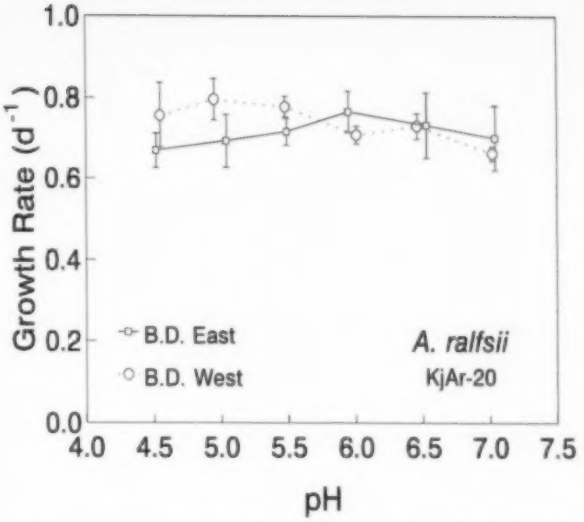

FIG. 2. Instantaneous growth rates as a function of initial experimental pH for the Lake Kejimkujik clone of *A. ralfsii* (KjAr-20) when grown in Big Dam Lake East (solid line) and Big Dam Lake West (broken line) media. Error bars represent ±2 SE about each mean growth rate.

This clone grew at >50% of maximum growth rate (usually approximately 0.7–0.8·d^{-1} for this species; Gensemer 1991a; Riseng et al. 1991; present study) at almost any pH. Overall, growth rates were not significantly affected by either medium type ($F_{2,45} = 1.74$, $p = 0.187$) or pH ($F_{5,45} = 2.09$, $p = 0.084$). In the artificial medium FRAQUIL, growth rates exceeded 0.6·d^{-1} at all pH values, and only one treatment group (pH = 6.0, growth rate = 1.07·d^{-1}) was significantly higher than the rest (one-way AOV: $F_{5,12} = 3.92$, $p = 0.024$). Growth rates in Plastic Lake media were also high at all pH values from 4.5 to 6.5 and then dropped significantly to 0.41·d^{-1} at pH 6.9 (one-way AOV: $F_{5,21} = 4.77$, $p = 0.004$). In contrast, growth rates in Lake Kejimkujik media showed no significant trend with pH and ranged from 0.76 to 0.80·d^{-1} in all treatments.

Additional pH tolerance experiments with the Lake Kejimkujik clone (KjAr-20) were run using water from Big Dam Lake in Kejimkujik Park, Nova Scotia. Growth rates in Big Dam Lake media ranged from 0.66 to 0.80·d^{-1} (Fig. 2) and exhibited no significant pH effect ($F_{5,25} = 1.76$, $p = 0.1599$). This was true regardless of whether cultures were grown in Big Dam Lake East or West media ($F_{1,24} = 2.75$, $p = 0.1100$).

As with the Lake Kejimkujik clone, the *A. ralfsii* isolate from Plastic Lake (PL-10) exhibited wide tolerance for pH (Fig. 3). When tested in either FRAQUIL or Lake Kejimkujik media, mean growth rates were high (ranging from 0.64 to 0.89) and showed no significant trend with pH. However, medium type ($F_{2,54} = 13.95$, $p < 0.0001$) and pH ($F_{5,54} = 3.63$, $p = 0.0066$) did significantly affect mean growth rates, but with no significant interaction ($F_{10,54} = 1.58$, $p = 0.1377$). This occurred because growth rates were affected by pH only in Plastic Lake media where they decreased approximately 60% at nominal pH ≥ 6.4 relative to maximum rates at pH 5.5–6.0.

In contrast, *A. formosa* (clone GB-15) was less tolerant of growth at low pH in all medium types (Fig. 4). Mean growth rates were strongly correlated with pH ($F_{3,24} = 123.55$, $p < 0.0001$) and were also dependent on medium type

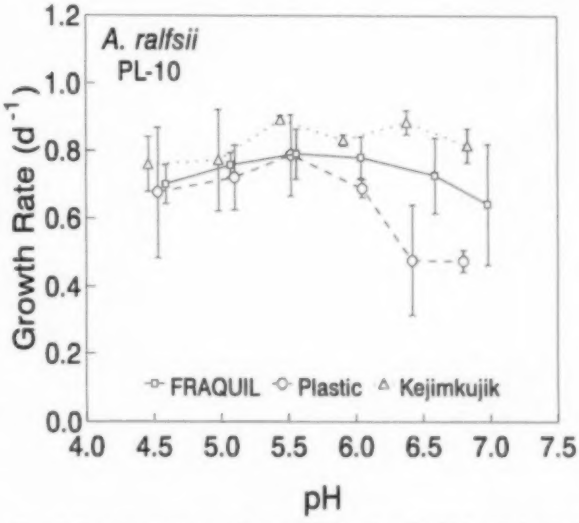

FIG. 3. Instantaneous growth rates as a function of initial experimental pH for the Plastic Lake clone of *A. ralfsii* (PL-10) when grown in FRAQUIL, Plastic Lake, and Lake Kejimkujik media. Error bars represent ±SE about each mean growth rate.

($F_{2,24} = 74.31$, $p < 0.0001$) and their interaction ($F_{6,24} = 41.723$, $p < 0.0001$). In FRAQUIL media containing 5 μmol EDTA·L^{-1}, growth rates increased with increasing pH, with minimum growth (0.05·d^{-1}) occurring at pH 5.5 and maximum growth (0.70·d^{-1}) occurring at pH 7.0 (Fig. 4A). Decreasing EDTA to 0.5 μmol·L^{-1} significantly affected pH tolerance relative to the higher EDTA experiments ($F_{1,24} = 18.4$, $p = 0.0002$) by stimulating growth at pH values ≤6.1 such that minimum growth occurred approximately 0.5 pH unit lower (at pH = 5.1) and by reducing growth at high pH relative to maximal rates at pH 6.1 (Fig. 4A).

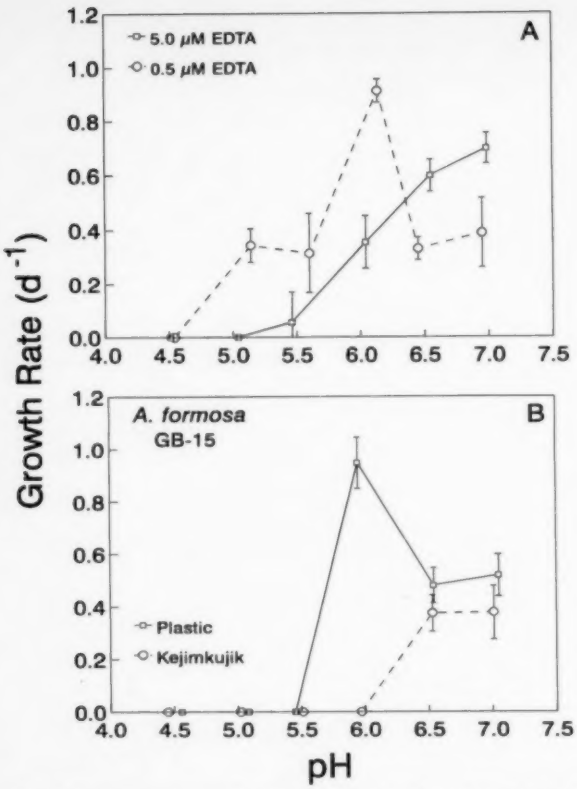

FIG. 4. Instantaneous growth rates as a function of initial experimental pH for the Lake Huron clone of *A. formosa* (GB-15) when grown in (A) FRAQUIL (solid lines represent 5.0 μmol EDTA·L^{-1} treatments; broken lines represent 0.5 μmol EDTA·L^{-1} treatments) and (B) Plastic Lake and Lake Kejimkujik media. Error bars represent ±2 SE about each mean growth rate.

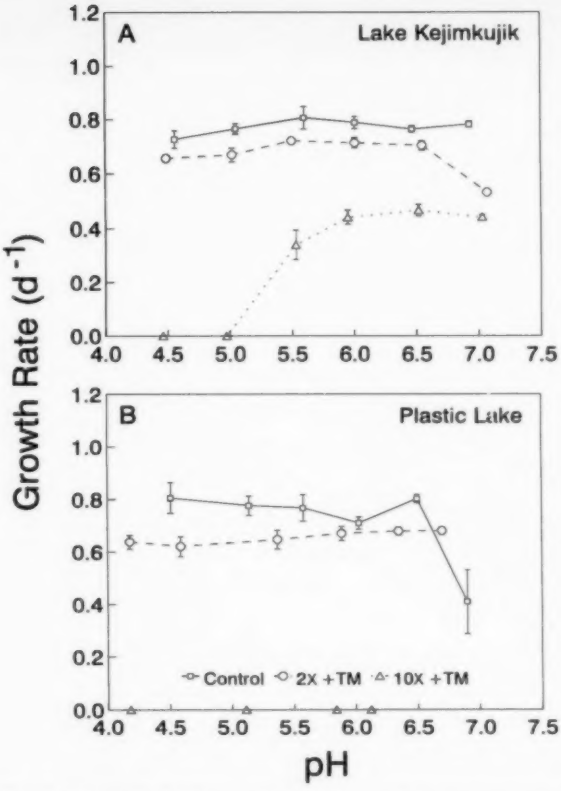

FIG. 5. Effects of mixed trace metal additions on instantaneous growth rates as a function of initial experimental pH for *A. ralfsii* (clone KjAr-20) when grown in (A) Lake Kejimkujik and (B) Plastic Lake media. Data labelled as "Control" represent media with no metal addition, and those labelled as "2× + TM" and "10× + TM" represent media with trace metals increased to two- to 10-fold higher than Lake Kejimkujik background concentrations, respectively (Table 3). Error bars represent ±2 SE about each mean growth rate.

Maximum growth rates for *A. formosa* occurred at pH 6.1 in Plastic Lake media, even though no growth was observed at lower pH (Fig. 4B). Growth in Plastic Lake media significantly decreased by 53% above pH 6.1 to modest rates of 0.48–0.52·d^{-1}, similar to both *A. ralfsii* clones (Fig. 1B, 3B). No growth occurred in Lake Kejimkujik media at any pH < 6.5, and growth rates at pH 6.5 and 7.0 were low (0.37–0.38·d^{-1}) compared with maximum rates in other medium types (Fig. 4B).

Metal Addition Experiments

Metal additions to Kejimkujik lake water media reduced overall population growth rates and affected pH tolerance by the Lake Kejimkujik clone of *A. ralfsii* (Fig. 5A). Both an AOV model including all data and one excluding data below pH 5.5 (to remove 0 variance cells resulting from no measurable growth in 10× + TM treatments) showed that metal addition ($F_{2,45} = 726.1, p < 0.0001$ and $F_{2,30} = 231.0, p < 0.0001$, respectively), pH ($F_{5,45} = 38.0, p < 0.0001$ and $F_{3,30} = 4.4$, $p = 0.0113$, respectively), and their interaction ($F_{10,45} = 30.0$, $p < 0.0001$ and $F_{6,30} = 7.3$, $p < 0.0001$, respectively) all affected population growth rates. As shown earlier (Fig. 1), no significant pH effects were observed in control treatments. Doubling metal concentrations significantly reduced overall growth rate by 14%, yet no significant pH effect was observed. In contrast, increasing ambient trace metal concentrations by 10× reduced growth at pH ≥ 6.0 to nearly 50% of control rates, significantly reduced growth further at nominal pH = 5.5, and prevented growth at pH < 5.5. The 10× metal additions were also sufficient to induce DOC precipitation in treatments of pH ≤ 5.0, as demonstrated by the formation of a brown floc minutes after metal addition to the media.

Mixed trace metal additions to Plastic lake water media had a greater negative effect on growth at high levels (Fig. 5B) and showed less overall pH dependence than in Lake Kejimkujik media experiments. With all data included, metal addition ($F_{2,45} = 237.5$, $p < 0.0001$) and a metal/pH interaction ($F_{10,45} = 2.84, p = 0.008$) significantly affected growth rates, but pH alone had no effect. The metal effect was largely a consequence of the 10× metal addition treatments where growth was eliminated at all pH levels tested. In the 2× metal additions at pH 6.7, growth rates were higher (0.68·d^{-1}) than in the absence of added metals at the same nominal pH (measured pH = 6.9; Fig. 5B). Considering only treatments of pH < 6.7 (and excluding 0 growth 10× metal addition treatments), however, 2× metal addition treatments in this medium

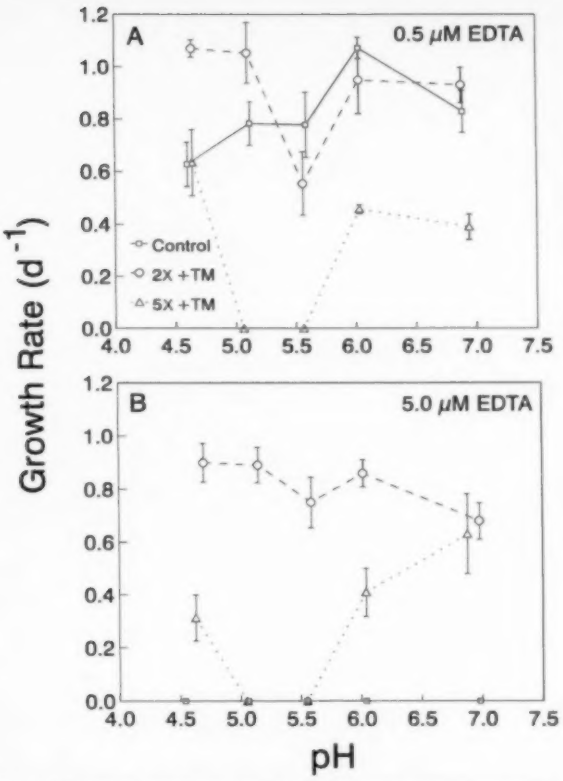

FIG. 6. Effects of mixed trace metal additions on instantaneous growth rates as a function of initial experimental pH for *A. ralfsii* (clone KjAr-20) when grown in FRAQUIL media containing (A) 0.5 μmol EDTA·L^{-1} and (B) 5.0 μmol EDTA·L^{-1}. Data labelled as "Control" represent media with no metal addition, and those labelled as "2× + TM" and "5× + TM" represent media with trace metals increased to two- to fivefold higher than Lake Kejimkujik background concentrations, respectively (Table 4). Error bars represent ±2 SE about each mean growth rate.

significantly decreased overall mean treatment growth rate relative to controls by 16% ($F_{1,26} = 27.0$, $p < 0.0001$).

Mixed trace metal additions to FRAQUIL media exerted more variable effects on pH tolerance by *A. ralfsii* (Fig. 6). Both metal addition ($F_{2,30} = 78.0$, $p < 0.0001$) and pH treatments ($F_{4,30} = 9.9$, $p < 0.0001$) as well as their interaction ($F_{8,30} = 5.8$, $p = 0.0002$) significantly affected growth rates in 0.5 μmol EDTA·L^{-1} media (Fig. 6A). As already observed (Fig. 1), growth rates exceeded 0.6·d^{-1} at all pH values in control media, but 2× + TM treatments significantly increased growth to 1.07·d^{-1} at pH 4.6 and to 1.05·d^{-1} at pH 5.1, yet decreased growth nearly 50% from these values at pH 5.6. Metal additions 5× in excess of Lake Kejimkujik background concentrations induced a much greater influence on pH tolerance by completely inhibiting growth at pH 5.0–5.6, even though significant growth occurred in treatments with either lower or higher pH. Furthermore, 5× metal additions at high pH significantly reduced growth >50% relative to controls at pH ≥ 6.0.

Identical metal addition and pH treatments using media containing 5.0 μmol EDTA·L^{-1} produced qualitatively similar results at both 2× and 5× metal addition levels, but did not grow in any control (= no metal addition) treatments (Fig. 6B). For these experiments, an AOV model which excluded control treatments (because of 0 variance in no-growth treatments) again showed that metal addition ($F_{1,20} = 114.8$, $p < 0.0001$) and pH treatments ($F_{4,20} = 4.8$, $p = 0.0068$) and their interaction ($F_{4,20} = 8.1$, $p = 0.0005$) significantly influenced growth rates. As in 0.5 μmol EDTA·L^{-1} media, growth rates in the 2× metal addition treatments were highest at pH ≤ 5.1 and lower at pH 5.6, but these differences were not statistically significant. The 5× metal addition treatments also completely inhibited growth at pH 5.0–5.6, and growth rates were extremely low at pH 4.6 and 6.0 (= 0.31 and 0.41·d^{-1}, respectively). Growth rates in the 2× and 5× metal addition treatments were nearly identical at pH 6.9 (Fig. 6B).

Discussion

Asterionella ralfsii was more broadly tolerant of pH variations over the range 4.5–7.0 than its congener *A. formosa*. Its wider tolerance was evident in various natural and artificial media with DOC of differing origin and concentration (Fig. 1–3). These results suggest that diatoms from acidic habitats may have relatively little physiological restriction on their growth near neutral pH whereas diatoms from circumneutral habitats are effectively incapable of growth at low pH (4.5–5.5). Furthermore, the pH tolerance of both *A. ralfsii* and *A. formosa* often depended on the concentration and nature of DOC present and on trace metal bioavailability. The present results do, however, generally support the classification of *A. ralfsii* as acidophilic compared with *A. formosa*, as discussed more fully below.

Genotypic variation among clones is a recognized source of variability in algal physiology (Gallagher 1982; Soudek and Robinson 1983). Such variation is still poorly characterized for most populations, however, and may confound attempts to compare clones from the same species. Our two *A. ralfsii* clones, which were isolated from geographically separate lakes with different chemical characteristics, performed similarly with respect to growth rate (Fig. 1, 3). Relatively uniform isolation conditions could have selected for similarly adapted clones regardless of the characteristics of the original host populations, but care was taken to conform both DOC (as EDTA) and pH of the initial isolation media (FRAQUIL) to those of the corresponding isolation site. It therefore seems more likely that strong genotypic adaptation to the differing chemical characteristics is not evident under our experimental conditions even though DOC is about 3× more concentrated in Lake Kejimkujik than in Plastic Lake (Table 2).

The proximate determinants of pH tolerance in our cultures could have included dissolved inorganic carbon (DIC) speciation and concentration, bioavailability of metals and inorganic nutrients, and direct H^+ toxicity. Both the speciation and concentration of DIC are strongly pH dependent (Morel 1983), and C limitation may occur in algal cultures at pH levels less than approximately 6 where total DIC concentrations are minimal (Williams and Turpin 1987). The tolerance of more acidic pH than 6 by *A. ralfsii* is inconsistent with limitation by DIC, but *A. formosa* might have been limited by DIC where growth rates suffered in some of the media at low pH (Fig. 4). However, DIC limitation cannot explain why low-pH growth by *A. formosa* in FRAQUIL could be improved by reducing the EDTA concentration (Fig. 4A). Furthermore, a similar limit to growth at low pH would be expected in both lake water media if only DIC limitation were important, but this limit, and the

pattern of the pH response, differed significantly (Fig. 4B). Although indirect, these observations suggest that DIC availability was not a deciding factor in the pH tolerance of either species. The same observations also refute the possibility that direct H^+ toxicity could have affected growth at low pH, particularly for *A. formosa*.

Clearly, media containing different types and/or concentrations of DOC can affect algal pH tolerance. Of several hypotheses used to explain phytoplankton responses to acidification (Stokes 1986), DOC is most likely to directly influence pH tolerance through changes in trace metal and/or nutrient bioavailability. Measurements of soluble reactive P and Si, the nutrients most likely to limit freshwater diatom growth (Wetzel 1983; Reynolds 1984), confirmed that concentrations were high in all control media (Table 2) and thus were unlikely to limit growth. Manipulations of pH and trace metals never reduced soluble reactive Si below control concentrations, but did reduce soluble reactive P concentrations in some treatments. For example, the highest metal additions to FRAQUIL and Plastic Lake media reduced soluble reactive P concentrations to <1 $\mu mol \cdot L^{-1}$ (means $\pm$ SE $= 0.17 \pm 0.17$ and 0.32 ± 0.15 $\mu mol \cdot L^{-1}$, respectively). Furthermore, $10\times$ trace metal additions induced DOC precipitation in Kejimkujik media at pH ≤ 5.1, concurrent with a reduction in soluble reactive to 0.8–0.4 $\mu mol \cdot L^{-1}$. No growth occurred in some of these high metal addition treatments (Fig. 5, 6), but such concentrations are highly unlikely to limit growth of *A. ralfsii* in vitro (Gensemer 1991b; R. W. Gensemer, unpubl. data). By elimination, therefore, it appears that metals are the likeliest proximate agents of pH response under the conditions studied here.

Our experiments suggested that one or more of the metals could be limiting to growth using standard metal concentrations, and this usually occurred at low pH. Growth of *A. formosa* at pH < 6.0 was inhibited by increasing EDTA concentration which, we presume, decreased trace metal activities (Fig. 4A). Metal chelation by natural DOC also may have reduced trace metal availability to *A. formosa* which did not grow below pH 6.5 in Lake Kejimkujik media but grew at pH as low as 6.1 in Plastic Lake media (Fig. 4B). The more concentrated DOC of Lake Kejimkujik would, again, be expected to provide greater metal-complexing capacity. Evidence of metal limitation was more direct for *A. ralfsii*, which benefited significantly from $2\times$ metal additions at pH 4.6 and 5.1 in 0.5 μmol EDTA$\cdot L^{-1}$ media (Fig. 6A), and exhibited maximal growth at low pH in $2\times$ metal additions using 5.0 $\mu mol \cdot L^{-1}$ media (Fig. 6B). Under the range of conditions studied here, the likeliest reason for the poor performance of *A. formosa* at low pH, compared with *A. ralfsii*, thus may be its relative inability to satisfy requirements for one or more trace metals. Conversely, *A. ralfsii*'s relatively poor growth at pH 6.9 in Plastic Lake media (Fig. 1, 3) was improved by a $2\times$ metal cocktail addition at similar pH (Fig. 5B). No such stimulation at high pH was observed in either of the other media. It would appear that, in certain natural waters such as Plastic Lake, trace metal limitation can also be a factor determining the upper limits of pH tolerance by *A. ralfsii*.

Despite the evidence for suboptimal availability of metals in some treatments, excessive availability was also induced, and this affected algal pH tolerance in some cases. For example, order of magnitude metal additions prevented growth at low pH in Lake Kejimkujik media (Fig. 5A). The highest ($5\times$) metal additions in FRAQUIL also induced toxicity at low pH, although growth recovered at pH 4.6 regardless of EDTA concentration (Fig. 6). In contrast, lower $2\times$ + TM additions significantly reduced growth rates in both Plastic and Kejimkujik lake water media but did not exert pH-dependent changes in growth (Fig. 5). Similar metal additions to FRAQUIL media had less of a toxic effect on *A. ralfsii*, yet also did not restrict its ability to grow over the entire pH range studied (Fig. 6). Therefore, seemingly minor increases in metal concentrations have the potential to inhibit algal growth in natural waters but may not affect pH tolerance. If metal additions are great enough, however, they can inhibit algal growth at certain, usually low, pH levels.

Metals are commonly considered to become increasingly toxic as pH falls, owing to greater concentrations of the most toxic chemical species at low pH (i.e. type II metals; Campbell and Stokes 1985). Metal toxicity occurred primarily at low pH in our experiments; thus, one or more of the metals in our mixture may have exhibited similar behaviour. This confirms earlier work (Gensemer and Kilham 1984) in which mixed metal toxicity in artificial media inhibited algal growth in some taxa, but only at low pH. Some metals, including Cd, Cu and, possibly, Al, can also become less bioavailable as pH declines, probably due to increased competition for binding sites with H^+ (i.e. type I metals; Campbell and Stokes 1985; Peterson and Healey 1985; Gensemer 1991a). That metals were limiting primarily at low pH in our experiments is consistent with type I metal behaviour because higher H^+ activities could, theoretically, hinder the acquisition of essential trace metals. The apparent consistency of our results with both types of metal behaviour may be a result of manipulating several metals simultaneously because an individual metal in trace metal mixtures is not always the most biologically reactive at all pH levels (B. E. Hickie et al. unpubl. data). Natural acidification often increases the concentration of several metals (LaZerte 1986; Mach and Brezonik 1989; Nelson and Campbell 1991); thus, our results point out the potential for variation in biological responses to the simultaneous manipulation of several chemical factors in natural waters.

Both natural (Sunda and Lewis 1978; Toledo et al. 1982; Sedlacek et al. 1983; Starodub et al. 1987) and artificial (Sunda and Guillard 1976; Morel et al. 1978; Petersen 1982; Starodub et al. 1987) DOC are widely believed to have an important influence on the bioavailability of metals to phytoplankton. Our FRAQUIL metal addition experiments (Fig. 6) demonstrated the complexing power of the synthetic chelator EDTA even at total C concentrations far below that contained in our natural lake water media (Table 2). The higher EDTA concentration (5 $\mu mol \cdot L^{-1}$) apparently made metals limiting to *A. ralfsii* at standard concentrations, but moderate metal additions ameliorated this effect (Fig. 6B). Curiously, however, 5 μmol EDTA$\cdot L^{-1}$ did not ameliorate the effect of the $5\times$ metal additions any better than the lower EDTA concentration. The complicated interactions of metals with the chelator and other inorganic components of the media, especially when added as a mixture, may account for this seeming anomaly.

Trace metal additions to natural lake water media provided a more easily understood response. In the relatively high DOC Lake Kejimkujik media, growth of *A. ralfsii* continued down to pH 5.5 even at $10\times$ addition levels (Fig. 5A). In contrast, growth was eliminated using the same $10\times$ addition at all the pH levels tested in Plastic Lake media (Fig. 5B). Relative differences in background concentrations of metals between the natural media should have been unimportant at the higher metal additions, and both lakes are similarly dilute in ionic strength. The results, therefore, appear to be a straightforward demon-

stration of the relative chelating powers of natural DOC in low-alkalinity waters.

pH Tolerance and Paleolimnology

In order to compare our results with predictions of algal pH tolerance from paleolimnological models, we will assume that maximum culture growth rate is analogous to an optimal distribution for the same species in nature. Models that reconstruct pH histories have been based traditionally on assignments of individual diatom species into one of five pH categories, as originally described by Hustedt (1939) (as presented in Charles et al. 1989): *alkalibiontic*, species occurring at pH values >7; *alkaliphilic*, species occurring at pH about 7, with widest distribution at pH >7; *indifferent* (=circumneutral), species with equal distribution about pH 7; *acidophilic*, species occurring at pH about 7, with widest distribution at pH < 7; and *acidobiontic*, species occurring at pH < 7, with optimum distribution at ≤ pH 5.5. Even though models based on these categories are being abandoned in favour of improved methods (see below), we will compare our results with these categories both for historical value and for interpreting the role of *A. ralfsii* in the Lake Kejimkujik pH reconstruction (Duthie 1989).

Based on growth rates, our results suggest that *A. ralfsii* is acidophilic in FRAQUIL and low-DOC lake water (Big Dam Lake East) media, as well as in high-DOC natural lake water media (Lake Kejimkujik and Big Dam Lake West). In these media, near-maximum growth occurred at any pH below 7, yet did not prefer any particular pH (Fig. 1–3). Survey data used in diatom-inferred pH reconstructions also classify natural populations of *A. ralfsii* (both nominate and *americana* varieties) as acidophilic in most cases (Table 5). *Asterionella ralfsii* instead conforms to an acidobiontic classification in Plastic Lake media owing to reduced growth at high pH (Fig. 1, 3). However, trace metal manipulations changed these apparent pH classifications. Alleviating trace metal limitation (2× + TM treatments) at high pH in Plastic lake water media makes an acidophilic classification more appropriate (Fig. 5B). In contrast, trace metal stress (e.g. in 5× and 10× metal addition treatments) makes pH-indifferent or alkaliphilic classifications often suitable owing to reduced growth at low pH (Fig. 5B, 6). Therefore, the assignment of this taxon to any particular pH category is highly dependent on growth medium chemistry, particularly with respect to trace metal availability.

Recent improvements in paleolimnological inference models such as canonical correspondence analysis (CCA) (Charles et al. 1989; Davis et al. 1990; Dixit et al. 1991) and weighted averaging regression (Birks et al. 1990; Kingston and Birks 1990) eliminate the need for pH category assignments by deriving specific weighted mean abundance distributions for individual taxa with respect to pH. Abundance-weighted mean pH distributions for *A. ralfsii* usually indicate maximal abundance in lakes of pH 5–5.5, although mean pH distributions differ in some regions (see Table 5: Kingston et al. 1989 and Sweets et al. 1989). Our experiments concur with the more common abundance-weighted mean pH values of 5–5.5 because maximum growth rates usually occurred at pH 5.5 regardless of medium type (Fig. 1–3). Natural populations of *A. ralfsii* also became abundant in Lake 223 (Experimental Lakes Area, Ontario) when pH was lowered to 5.56 but became absent after pH was further reduced (Findlay and Kasian 1986). In our experiments, the highest metal additions often changed the pH at which maximum growth rates occurred (Fig. 5, 6). While regional variations in apparent pH classifications for natural populations may depend on reference lake selection and the pH distribution of lakes within each reference set (Kingston et al. 1989), our results suggest that pH references for this taxon can also depend on trace metal bioavailability. That DOC seems to correlate with the natural distribution of *A. ralfsii* with respect to pH (Charles et al. 1990) also suggests that trace metal bioavailability may be an important factor in the distribution of this taxon in acidic lakes.

If, as our data suggest, *A. ralfsii* can be categorized as acidophilic when grown in Kejimkujik and Big Dam West lake water, then our results corroborate its classification in the Lake

TABLE 5. Literature pH classifications for natural assemblages of *Asterionella* in terms of Hustedt (1939) pH categories and abundance-weighted mean (AWM) pH distributions.

Species	pH category	AWM	Reference
A. ralfsii W. Smith	Acidophilic		Eloranta 1988
	Acidophilic	4.94	Birks et al. 1990
	Acidophilic	5.30	Huttunen and Turkia 1990
A. ralfsii var. *americana*	Acidophilic	5.1	Charles 1985
Körn.	Acidophilic	5.5	Davis and Smol 1986
	Acidophilic		Duthie 1989
		6.10	Kingston et al. 1989
	Acidophilic	4.7	Sweets et al. 1989
		5.53	Davis et al. 1990
		5.57	Dixit et al. 1991
A. ralfsii var. *americana*			
<45 μm	Acidobiontic		Charles et al. 1990
		4.9	S. S. Dixit et al., unpubl. data
>45 μm	Acidophilic		Charles et al. 1990
		5.4	S. S. Dixit et al., unpubl. data
A. formosa Hass.	Alkaliphilic	7.4	Charles 1985
		6.70	Kingston et al. 1989
	Alkaliphilic	6.72	Birks et al. 1990
	Alkaliphilic	6.16	Huttunen and Turkia 1990
		6.84	Dixit et al. 1991
		7.3	S. S. Dixit et al. unpubl. data

Kejimkujik pH reconstruction (Duthie 1989). However, if decreased humification has occurred in Lake Kejimkujik since 1950, it is difficult to judge whether this categorization of *A. ralfsii* should remain constant over the entire period represented by this core. For example, an acidobiontic classification could be more appropriate for recent populations if DOC concentrations are changing to the point where high-pH metal limitation could occur as in our Plastic Lake experiments (Fig. 5B). A change toward more "acidobiontic" characteristics would also explain why the surface sediment assemblages infer a pH lower than recent measurements for Lake Kejimkujik (Duthie 1989).

Similar to Lake Kejimkujik, Little Echo Pond (mid-1980's epilimnetic pH = 4.28) apparently has not acidified significantly since preindustrial times, but this interpretation also largely depends on the pH preferences of the *A. ralfsii* (Charles et al. 1990). In the Adirondacks, short cell-length forms of *A. ralfsii* var. *americana* (<45 μm) usually inhabit small humic lakes (including Little Echo Pond; DOC = 12.5 $mg \cdot L^{-1}$) and are considered acidobiontic whereas longer forms (>45 μm) inhabit clearer lakes and are classified as acidophilic (Charles et al. 1990). Throughout our experiments, both our clones exhibited "long" cell length by this classification (KjAr-20 mean cell length = 66.8 μm, SE = 0.08; PL-10 mean cell length = 70.5 μm, SE = 0.05), so it is unclear whether our presumably acidophilic clones are the same taxonomic entity as populations in Little Echo Pond. However, trace metal bioavailability can affect both pH tolerance (present study) and cell length (Gensemer 1990) in *A. ralfsii* var. *americana*. The lower apparent pH preference of the Little Echo Pond populations thus may reflect the chemical characteristics of this lake rather than a unique ecological preference of a different taxon classified by size. Furthermore, size-based diatom classifications are questionable because changes in cell length may also reflect differences in cell cycle stages (Round et al. 1990) which are as yet poorly described in *Asterionella* (Mann 1988).

A pH-indifferent (i.e. circumneutral) classification for *A. formosa* is consistent with our data, but given the restricted pH range of our experiments (4.5–7), we also cannot reject an alkaliphilic classification as is often used in paleolimnological studies (Table 5). Weighted average pH values for natural assemblages (Table 5) are also usually consistent with our maximal growth rates at pH 7.0, except when grown in low-DOC artificial media (Fig. 4A). Similar to our study, a Lake Michigan isolate of *A. formosa* would not grow below pH 6 when grown in FRAQUIL (Riseng et al. 1991). The same species also grew poorly at pH 5 in a medium with higher alkalinity and ionic strength (WC), although growth increased to control rates if trace metal concentrations were reduced 10–100× (Gensemer and Kilham 1984). Therefore, even though the tolerance of specific pH levels by *A. formosa* can be influenced by DOC and/or trace metal bioavailability, none of these effects probably influences its assignment to pH categories or distributions.

In summary, our results suggest that *A. ralfsii* is not limited by DIC availability, H^+ toxicity, or major nutrient limitation over the range of pH studied here. The circumneutral diatom *A. formosa* was clearly less acid tolerant than *A. ralfsii*, and the likeliest physiological explanation for the difference appeared to be the relative inability of *A. formosa* to satisfy trace metal requirements at lower pH. The pH tolerance of *A. formosa*, and at times *A. ralfsii*, could be modified by DOC and, probably, by metal concentrations. An important implication is that these indicator species will respond differently to pH variations depending on other changes in lake water chemistry, notably in DOC and metal loading from acidified catchments. The assignment of either *Asterionella* species to a particular pH category or distribution thus may not relate only to an inherent biological property of that taxon, but may also strongly depend on ambient chemical conditions. Such assignments still are based purely on empirical correlations of natural abundances with water chemistry data and thus do not necessarily represent true physiological preferences for these specific conditions (Davis and Smol 1986). Because of the complex interactions between lake acidity, DOC concentration, and trace metal bioavailability, DI pH reconstructions for humic lakes need to be viewed with caution, particularly if they use pH category based models. CCA-based inference models can better cope with interactions among environmental factors (Dixit et al. 1991), but our understanding of the specific physiological and ecological responses of diatoms to these conditions should also be improved.

Acknowledgements

We thank Chief Warden J. Wentzel at the Kejimkujik National Park and D. Harris of the Canadian Parks Service for logistical support and permission to collect at Kejimkujik Park and B. D. LaZerte at the Ontario Ministry of the Environment for access to Plastic Lake, Ontario. We are grateful to J. Kerekes and R. Tordon for logistic support of our work at Kejimkujik Park and for providing water chemistry data and to S. S. Dixit for supplying unpublished data. We also appreciate N. Hopkins and R. Elgood for laboratory assistance, S. McIntyre for help with taxonomic identification, and R. C. Playle for reading an early draft of this manuscript. Helpful suggestions for the manuscript were also provided by B. F. Cumming, D. L. Findlay, H. Kling, and J. P. Smol. This work was supported by a strategic grant (STR0045063) from the Natural Sciences and Engineering Research Council of Canada awarded to R.E.H.S., H.C.D., and S.L.S.

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Growth Rate Variation and Larval Survival: Inferences from an Individual-Based Size-Dependent Predation Model

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Rice, J. A., T. J. Miller, K. A. Rose, L. B. Crowder, E. A. Marschall, A. S. Trebitz, and D. L. DeAngelis. 1993. Growth rate variation and larval survival: inferences from an individual-based size-dependent predation model. Can. J. Fish. Aquat. Sci. 50: 133–142.

We used an individual-based Monte Carlo simulation model to explore how changes in the mean and variance of growth rates of individuals in a larval fish cohort interact with size-dependent predation to affect the number and characteristics of individual survivors. Small changes in initial cohort mean growth rate can change survival over the first 60 d of life 10- to 30-fold. But when variance in growth rate among individuals is high, survival can be substantially higher than expected from the initial mean cohort growth rate. Selection for faster-growing individuals becomes stronger with increasing variance and increasing predation rate. In some cases, >80% of the survivors may come from the upper 25% of the initial growth rate distribution, and the mean growth rate of the survivors may exceed twice the initial mean growth rate. When individual growth rates change from day to day rather than remaining constant, the contribution of atypical individuals is accentuated even further. Counterintuitively, most of the selection for faster-growing individuals happens only after the majority of mortality has already taken place. These results suggest that interactions between individual variability and selective mortality may have important cohort-level implications for survival in fishes.

Nous avons utilisé un modèle de simulation Monte Carlo basé sur les individus pour explorer comment les changements de la moyenne et de la variance des taux de croissance d'individus dans une cohorte de larves de poisson interagissent avec la prédation en fonction de la taille pour influer sur le nombre et les caractéristiques des individus survivants. De petits changements observés dans la cohorte initiale signifient que le taux de croissance peut changer le taux de survie pour les premiers 60 jours de la vie par un facteur de 10 à 30. Quand la variance du taux de croissance observée chez les individus est élevée, le taux de survie peut être substantiellement plus élevé que celui qui est prévu à partir du taux de croissance initial moyen de la cohorte. La sélection favorisant les individus croissant plus rapidement devient plus forte avec une variance et un taux de prédation en progression. Dans certains cas, plus de 80% des survivants peuvent provenir des 25% supérieurs de la distribution initiale du taux de croissance, et le taux de croissance moyen des survivants peut dépasser deux fois le taux de croissance initial moyen. Quand les taux de croissance des individus changent de jour en jour au lieu de demeurer constants, la contribution des individus atypiques est accentuée encore davantage. Contrairement à ce que l'on pourrait croire intuitivement, la plus grande partie de la sélection, dans le cas des individus croissant rapidement, n'intervient qu'après que la plus grande partie de la mortalité ait eu lieu. Ces résultats suggèrent que les interactions entre la variabilité individuelle et la mortalité sélective peuvent avoir d'importantes implications au niveau des cohortes pour la survie des poissons.

Received September 24, 1990
Accepted September 2, 1992
(JA734)

Reçu le 24 septembre 1990
Accepté le 2 septembre 1992

Mechanisms governing survival, and ultimately selection, operate at the level of the individual. Often, the mortality sources that regulate survival are selective, so that some individuals may be more likely to survive than others, based on differences in some physical, behavioral, or physiological characteristics. When the amount of variation among individuals is low, or the selective forces are weak, models that are based on population or cohort averages are appropriate to represent the mechanisms operating to produce the aggregate population parameters we observe (Lotka 1925; Ricker 1954; Beverton and Holt 1957; Cushing 1975; Hutchinson 1978). However, when individuals vary substantially in characteristics important to survival, our understanding of

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mechanisms controlling cohort recruitment and other population-level responses may be enhanced by explicitly considering the effects of individual variability (Huston et al. 1988; Caswell 1989; Crowder et al. 1992).

Recent studies suggest that surviving larval and juvenile fish typically are not drawn at random from a cohort. In some cases, larvae hatching earlier are favored over younger larvae (Peterson and Wroblewski 1984; McGurk 1986), while in others, younger larvae are favored over older ones (Methot 1983; Crecco and Savoy 1985; Rice et al. 1987b). Large individuals may exhibit higher survival than small individuals within and among years (Post and Prankevicius 1987; Henderson and Cass 1991), and several studies suggest that faster-growing larvae may be favored over slower-growing individuals (Rosenberg and Haugen 1982; Rice et al. 1987b).

For larval and juvenile fishes, body size appears to be an important variable affecting survival. Most fishes grow 2–3 orders of magnitude in mass during the first few months of life (Werner and Gilliam 1984), and some of the major sources of larval mortality (predation and starvation) are strongly size dependent (Gutreuter and Anderson 1985; Rice et al. 1987a; Miller et al. 1988; Luecke et al. 1990).

Growth rate may be an important determinant of survival, especially when size-dependent mortality is important. For example, several studies suggest that relatively small reductions in mean growth rate during the early life history may cause cohort survival to decrease 1–2 orders of magnitude simply by prolonging the period of vulnerability to mortality sources (Chambers and Leggett 1987; Houde 1987, 1989; Pepin 1990). Growth rate variation among individuals within a cohort may also have significant effects. Growth rate and size often vary substantially among individuals within a cohort. Consequently, we might expect some individuals to differ markedly from the average fish in response to size-dependent mortality, causing the combined outcome of individual interactions to differ substantially from that for the average individual (Sharp 1987).

We used an individual-based Monte Carlo simulation model to explore how changes in the mean and variance of growth rates of a larval fish cohort might interact with size-dependent predation to affect the number and characteristics of individual survivors. In particular, we asked the following questions: How might an individual larva's probability of surviving size-dependent mortality be affected by its growth rate? We expect that variance in growth rates among individuals will lead to nonrandom survival among individuals, but what are its effects on cohort survival and the sizes and growth rates of survivors?

We based our model on the predator–prey interaction between alewife (*Alosa pseudoharengus*) and larval/juvenile bloater (*Coregonus hoyi*) because this interaction is well quantified. Rice et al. (1987b) showed that bloater recruitment in Lake Michigan was strongly influenced by size- or growth-rate-dependent mortality during the first 1–2 mo of life. Luecke et al. (1990) demonstrated experimentally that predation by juvenile alewife on larval bloater was strongly size dependent and may be a major source of this mortality. However, because size-dependent relationships appear to be relatively common among fishes (Miller et al. 1988), the patterns we report may have broad relevance for fishes in general.

Model Description

The model we developed tracks the daily growth and survival of individuals comprising a larval bloater cohort as they are exposed over 60 d to size-dependent predation by yearling alewife. Predator size was held constant at 90 mm, a representative size for yearling alewife, so that the effects of growth rate variation could be evaluated without the confounding effects of changing predator size.

In the model, all larvae start at a length of 12 mm, the typical size at which bloater larvae first become exposed to alewife in Lake Michigan (Rice et al. 1987b). In the initial series of simulations, each individual's growth rate was held constant over time so that the effects of variation among individuals could be readily evaluated. However, in nature, individual growth rates are unlikely to remain constant, so we conducted additional simulations to evaluate the effects of temporal variability in growth rates of individuals.

The probability of an individual fish being eaten on a given day (vulnerability) is the product of three probabilities: being encountered by a predator, being attacked if encountered, and being captured if attacked (Gerritsen and Strickler 1977). Probability of capture given an attack declines strongly with increasing prey size for yearling alewife feeding on larval and juvenile bloater (Luecke et al. 1990) as is typical of fish predators preying on larvae, but the other two probabilities have not been measured. The two most important factors determining encounter rate, predator reactive distance and swimming speed (Gerritsen and Strickler 1977), are both functions of predator size and therefore remain constant in our simulations, regardless of prey size. When the third factor, prey swimming speed, is much less than predator speed as we might expect for larval bloater and yearling alewife or for other relatively large fish predators on larvae, the encounter rate remains nearly constant, increasing only slightly with prey size (Gerritsen and Strickler 1977). Alewife readily eat both larval and juvenile bloater and even prefer them to zooplankton (Luecke et al. 1990), so it also seems unlikely that probability of attack would change noticeably over the range of prey sizes occurring in our simulations. Therefore, for our simulations, we set the daily probability of prey being both encountered and attacked as a constant.

The combined result of these probabilities is monotonically declining vulnerability with increasing prey size, which is a typical pattern for many larval fish predator–prey interactions (Bailey and Houde 1989). Some species exhibit lower vulnerability in early life stages (e.g. eggs or uneyed yolk sack larvae) because encounter rate is substantially reduced by their low visibility and/or low motility (e.g. Brownwell 1985; Folkvord and Hunter 1986). This may cause a slight "hump" in the vulnerability function at very small sizes, but even for these cases, vulnerability is a declining function over much of the early ontogeny. For relatively small, slow-moving, or ambush predators (e.g. chaetognaths, jellyfish), a more pronounced dome-shaped vulnerability function would be expected (Greene 1986; Bailey and Houde 1989). However, the assumption of constant probability of encounter and attack is appropriate for bloater larvae and juveniles preyed upon by yearling alewife and also produces an overall pattern of size-dependent vulnerability that is applicable to many other large, fast-swimming predators feeding on all but the smallest sizes of larvae.

The value used for the daily probability of encounter and attack governs the intensity of predation in the simulation. Clearly, changes in intensity of predation (e.g. changes in predator density) will have major effects on larval survival. Our intent was to evaluate the extent to which changes in mean and variance of larval growth rate would modify this survival rate. Therefore, we conducted all the individual-based model simulations using one value for daily probability of encounter and attack, 0.2, that gave us substantial mortality but still left

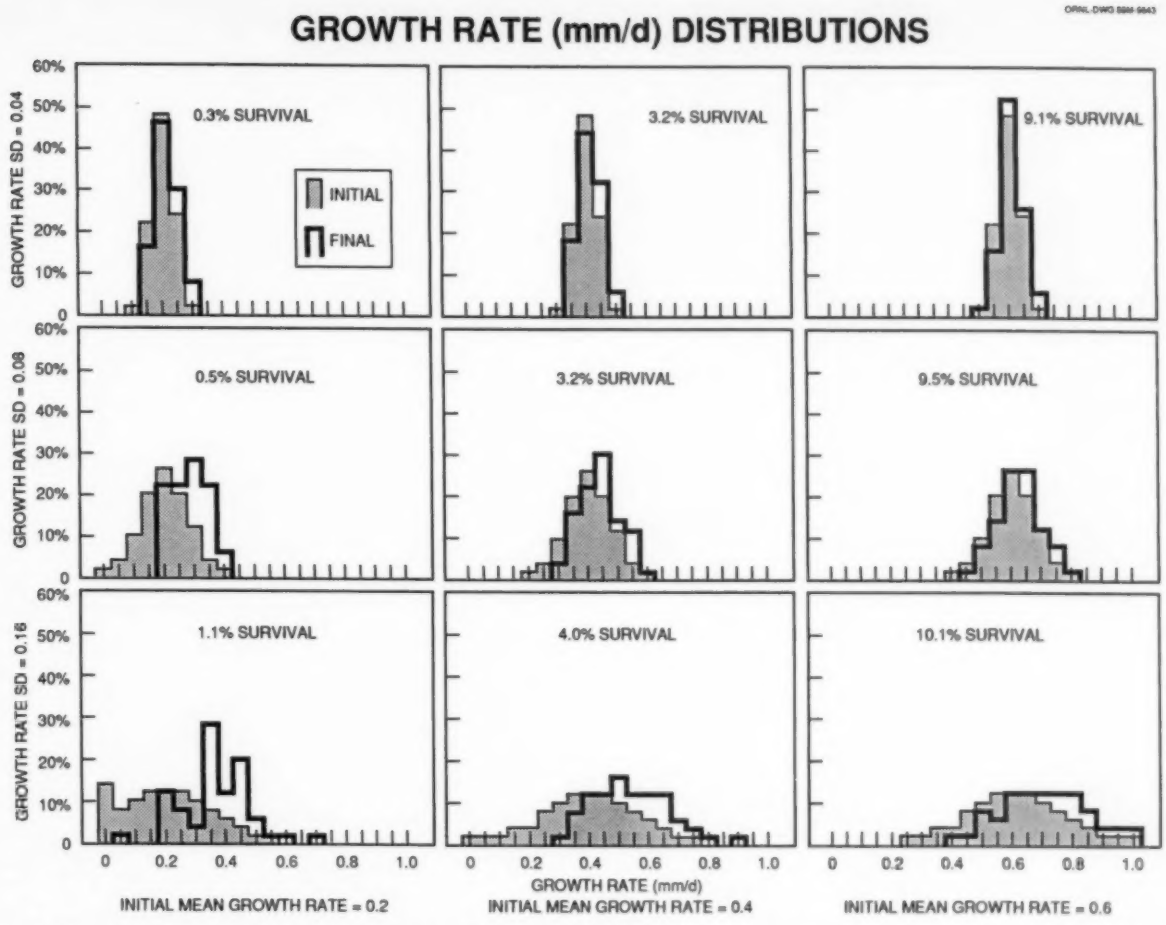

FIG. 1. Growth rate frequency distributions for cohorts of larval bloater having three initial mean growth rates (0.2, 0.4, 0.6 mm/d) and three levels of variability in growth rate among individuals (SD = 0.04, 0.08, 0.16 mm/d) before and after 60 d of size-dependent predation by 90-mm alewife. Values in each cell indicate the percent cohort survival after 60 d of predation.

enough survivors after 60 d for analysis. We assumed that the probability of multiple encounters and attacks on a single day was small, and therefore, we did not explicitly include it. On each day of a simulation, each fish was assigned a number drawn at random from a uniform distribution between 0 and 1; if the number was less than or equal to the probability of encounter and attack, an attack occurred. Whether the individual died or lived to the next day was determined by comparing a second random number beween 0 and 1 to the individual's probability of capture. Probability of capture, given an attack, was determined using the relationship between capture success and the predator–prey size ratio for 90-mm alewife preying on larval and juvenile bloater (probability of capture = $-0.33 + 0.15 \cdot$(predator length/prey length); Luecke et al. 1990); capture probability was set to 0 for bloater >40.9 mm, the size at which capture success reached zero. At the end of each time-step, each surviving individual grew to a new size determined by its growth rate and was then exposed to predation again on the next day. We followed the numbers, size distribution, and growth rate distribution of surviving individuals throughout each simulation.

Model Simulations and Results

Our initial series of simulations was designed to examine the hypothesis that when mortality is size dependent, both increased mean growth rate and increased variance in growth rate among individuals will increase cohort survival and that survivors will be drawn nonrandomly from the cohort. We conducted nine simulations using three mean growth rates (0.2, 0.4, and 0.6 mm/d), each with three different levels of variance in growth rate among individuals (SD = 0.04, 0.08, and 0.16 mm/d). Observed means and variances of growth rates for Lake Michigan bloater fall near the middle of these ranges; in the early 1980s, Lake Michigan larval bloater growth rates typically ranged from 0.25 to 0.75 mm/d (Rice et al. 1987b).

In these simulations, each larva was assigned a growth rate drawn at random from a normal distribution with the specified mean and variance and maintained this assigned growth rate throughout the entire simulation. Growth rates were constrained to the range of 0–1.0 mm/d; individuals drawing a growth rate below or above these bounds were assigned a growth rate of 0 or 1.0 mm/d, respectively. Several additional simulations were

TABLE 1. Means and 95% confidence intervals for percent survival of a larval fish cohort from 60-d simulations of the predation model. Each combination of growth rate mean and variance was repeated 30 times, with different random number seeds. Initial cohort size was 4000 larvae for each run. All means were significantly different from one another except for the low and intermediate variance simulations at high initial growth rate.

	Initial mean growth rate		
Growth rate SD	0.2 mm/d	0.4 mm/d	0.6 mm/d
0.04	0.362 ± 0.037	3.118 ± 0.096	9.538 ± 0.185
0.08	0.536 ± 0.033	3.323 ± 0.073	9.666 ± 0.151
0.16	1.124 ± 0.068	4.155 ± 0.010	10.068 ± 0.133

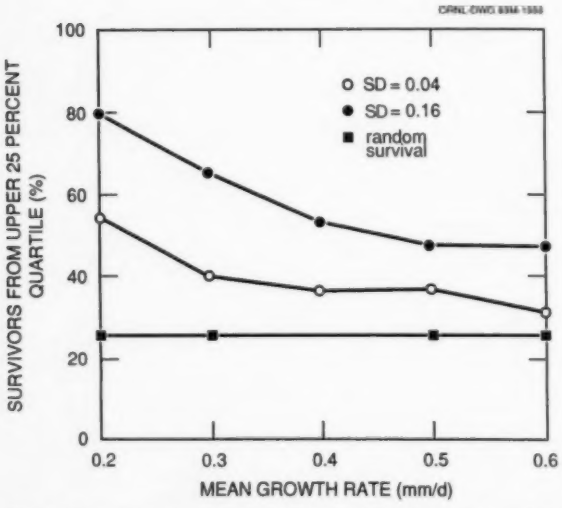

FIG. 2. Percentage of survivors after 60 d of size-dependent predation that came from the upper 25% of the initial growth rate distribution, plotted as a function of initial mean growth rate of the cohort, with high (SD = 0.16) and low (SD = 0.04) variability in growth rate among individuals. Level line indicates expected percentage if survival was random.

run at mean growth rates of 0.3 and 0.5 mm/d with high or low variance.

Most simulations were started with 4000 individuals, to impose a reasonable limit on computing time. In a few runs which exhibited very low survival, we used up to 10 000 individuals. The daily probability of encounter and attack was set at 0.2. We also repeated each of the nine initial runs with this probability set to zero, so we could compare the size distribution of survivors with and without predation; these runs were initiated with only 1000 individuals because there was no mortality.

Changes in the mean and variance in growth rate had substantial effects on survival and on the growth rate distribution of survivors (Fig. 1). Survival increased 10- to 30-fold with increasing mean growth rate. Survival also increased up to fourfold with increasing variance in growth rate, especially when the initial mean growth rate was low.

To evaluate the significance of these changes in survival, we ran 30 simulations for each growth rate mean and variance combination and calculated 95% confidence intervals for the estimates of survival (Table 1). These results indicate that the changes in survival we observed with increasing variance or initial mean growth rate are highly significant.

In these nine initial runs, individual fish maintained the constant growth rate assigned to them at the beginning of the simulation. Thus, changes in mean growth rates of the survivors resulted from selective mortality rather than from changes in the growth rates of individuals. As mean growth rate decreased and variance increased, the growth rate of surviving individuals became increasingly different from the initial average for the cohort, indicative of selection for faster-growing individuals. For example, at the lowest growth rate and highest variance, 80% of the individuals surviving 60 d came from the upper 25% of the initial growth rate distribution (Fig. 2). Even at the highest growth rate, almost 50% of the survivors came from the upper quartile when variation in growth rate among individuals was high (Fig. 2). When initial mean growth was low, and mortality was therefore high, even the lowest level of variability was sufficient to induce fairly strong selection, with more than 50% of the survivors coming from the upper 25% of the initial growth rate distribution. In every case the distribution of growth rates of survivors was different from what would have been expected if we had modeled the population using a single mean growth rate value. Clearly, if growth rate variability and size-dependent mortality interact in natural populations, survivors will not be drawn at random from the initial growth rate distribution.

The size-distribution of survivors in our simulations also reflects the interaction between size-dependent predation and the mean and variance of growth rates. As mean growth rate decreased and variance increased, survivors from cohorts subjected to predation were much larger than those from cohorts which experienced no predation (Fig. 3). When variation in growth rate among individuals was high, the mean size of individuals surviving predation was 5–10 mm (about 20%) greater than when growth rate variation was low.

Counterintuitively, in our simulations, the period of highest mortality was not coincident with the period of strongest growth rate selection. Mean growth rate changed little during the first few days when mortality was highest, and more than half the change in mean growth rate occurred during the last 1.5% of the mortality (Fig. 4). This asymmetry resulted because selective mortality was not acting directly on variability in growth rate, but on variability in size, which takes time to develop. Initially, fast- and slow-growing individuals were the same size and had the same high vulnerability to predation. While most survivors tended to be drawn from among the faster-growing individuals, most of the faster-growing fish died, along with their slow-growing counterparts, during this early period. However, because the size of fast-growing individuals was increasing rapidly, they experienced a more rapid decline in the daily probability of dying than did slow-growing individuals (Fig. 5a). Because these daily probabilities are compounded, the probability of surviving to day 60 increased much more

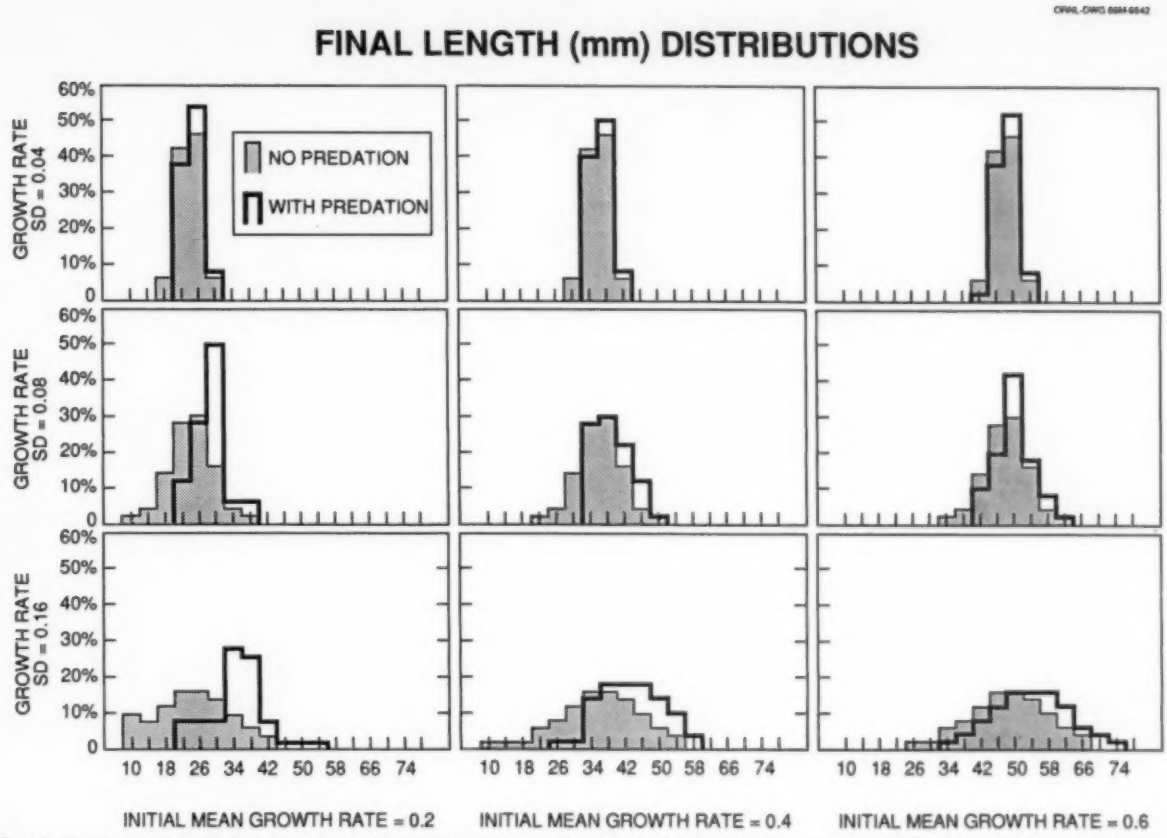

FIG. 3. Size frequency distributions for cohorts of larval bloater having three initial mean growth rates (0.2, 0.4, 0.6 mm/d) and three levels of variability in growth rate among individuals (SD = 0.04, 0.08, 0.16 mm/d) after 60 d with and without size-dependent predation by 90-mm alewife.

quickly for fast-growing individuals than for slow-growing fish (Fig. 5b). For example, after 10 d, an individual growing at 0.6 mm/d would have a 31% chance of living to day 60, while a fish growing at 0.2 mm/d would have only a 1.6% chance. After 30 d, the fast-growing fish would be 30 mm long and have an 83% chance of surviving, while the slow-growing individual would have grown to only 18 mm and have only a 14% chance of living to day 60.

The intensity of predation (i.e. encounter rate) will obviously have a major effect on the number of larvae surviving. However, higher predation mortality should also cause the effects of mean and variance in growth rate on characteristics of survivors to become even more pronounced because selective mortality is the cause of these changes. Our individual-based simulations were conservative in this regard. In order to have adequate numbers of survivors for analysis in all simulations, and work within the constraints of available computer memory and speed, we had to use a probability of encounter and attack that resulted in survival rates 1–3 orders of magnitude higher than typical estimates for fishes in the field (Forney 1976; Ware and Lambert 1985; Houde 1987; Taggart and Leggett 1987).

In contrast with our individual-based simulation model, partial differential equation (PDE) analytical models can incorporate more realistic mortality rates because they are not constrained by cohort size. Comparable PDE models cannot be developed for many individual-based simulation models, but we were able to formulate a PDE directly analogous to the simulations described above (Appendix). We used this model to evaluate the effect of predation rate on mean growth rate of surviving larvae for a cohort having an initial mean growth rate of 0.6 mm/d and high variance. In our individual-based simulations using a daily probability of encounter and attack of 0.2, the mean growth rate of survivors increased only 0.09 mm/d over 60 d because survival was so high (10.1%). When we used the PDE with a probability of encounter and attack of 0.7 which produced a more realistic cohort survival of 0.07%, the mean growth rate of survivors increased by 0.2 mm/d. Thus, substantial selection for faster-growing individuals can occur provided sufficient variability exists among individual growth rates and the magnitude of size-dependent mortality is sufficient to impose selection. The patterns observed in our relatively conservative simulations would be further accentuated at more realistic encounter rates. Note, however, that this selection may be somewhat less at high than at low initial mean growth rates because in the former case, a larger proportion of individuals will reach less vulnerable sizes earlier.

In the simulations discussed above, individual growth rates remained constant. A more complex, but potentially more realistic, assumption would allow growth rates of individuals to vary from day to day. We modeled this process as a random walk with "memory," where individuals possessing an above-average growth rate on day t would have an increased proba-

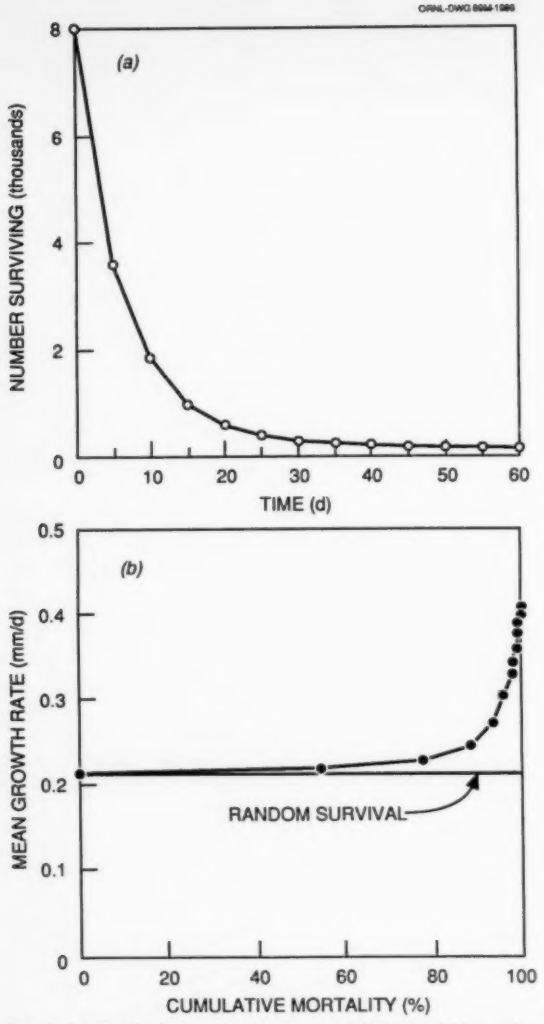

FIG. 4. (a) Survival curve for a cohort of 8000 larval bloater having an initial mean growth rate of 0.2 mm/d and high variability in growth rate among individuals (SD = 0.16 mm/d), when exposed to size-dependent predation by 90-mm alewife (daily probability of encounter and attack = 0.2) for 60 d. (b) Change in mean growth rate of surviving individuals from this same cohort as cumulative mortality of the cohort increases. Level line indicates expected result (no change in mean growth rate) if survival was random.

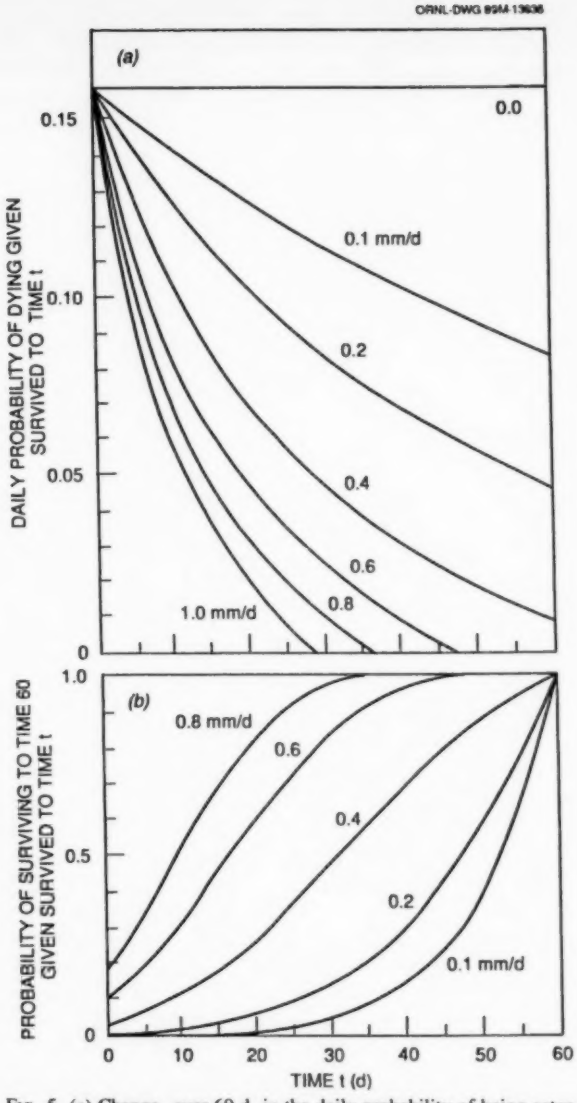

FIG. 5. (a) Change, over 60 d, in the daily probability of being eaten for larval bloater growing at different rates (0.0–1.0 mm/d) while exposed to size-dependent predation by 90-mm alewife, using the probability of capture equation from Luecke et al. (1990) and a probability of encounter and attack = 0.2. (b) Change, over 60 d, in the probability of surviving to day 60, given they are still alive at time t, for larval bloater growing at different rates while exposed to size-dependent predation by 90-mm alewife.

bility of achieving an above-average growth rate on day $t + 1$, and slow-growing larvae on day t would be more likely to have a below-average growth rate on day $t + 1$. We performed a simulation to test the hypothesis that the increased variance in average growth rates among individuals caused by such a random walk would have an ever greater effect on the number and size distribution of survivors than when individual growth rates were held constant. In this simulation the initial growth rate of each individual was drawn at random from a normal distribution with mean growth rate = 0.4 mm/d and SD = 0.08. On subsequent days, each individual's growth rate was drawn at random from a normal distribution of growth rates with the same variance (SD = 0.08), but centered on the individual's growth rate from the previous day. Growth rates were limited to the range of 0–0.8 mm/d; in cases where an individual's growth rate exceeded these limits, it was set equal to the limit. This kept the boundaries symmetrical around the initial mean growth rate, so that observed changes in mean growth rate during the simulation could be attributed completely to selective mortality, rather than random drift. The simulation was run both with and without predation (probability of encounter and attack = 0.2 or 0/d), and each of these was repeated with the growth rate of each individual held constant throughout the simulation (as in the previous simulation shown in the center panel of Fig. 1 and 3), for comparison.

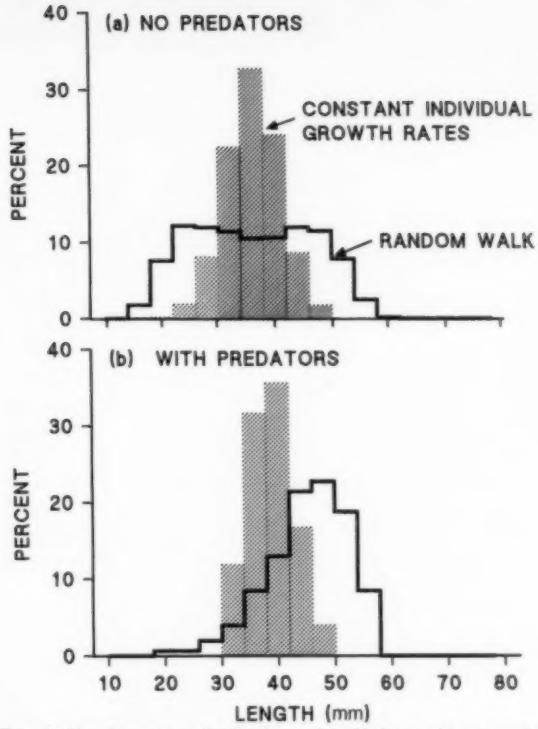

FIG. 6. Size frequency distributions after 60 d (a) without predation and (b) with size-dependent predation by 90-mm alewife for two cohorts of larval bloaters; one with constant individual growth rates (as in simulation shown in center panel of Fig. 1 and 3) and the other with individual growth rates that vary daily in a random walk between 0 and 0.8 mm/d. Both cohorts started with an initial growth rate of 0.4 mm/d and medium variability among individuals (SD = 0.08 mm/d).

When individual growth rates were allowed to vary daily in a random walk, the final size distribution after 60 d was substantially more variable than when individual growth rates were held constant, even in the absence of predation mortality (Fig. 6a). When size-dependent predation occurred, increased growth rate variability led to an increase in both the number and average size of survivors; 3.9% of the fish from the initial cohort survived to day 60 and attained a mean length of 45.3 mm in the random walk simulation, while only 2.5%, averaging 38.9 mm on day 60, survived from the constant growth rate simulation (Fig. 6b). The mean growth rate of survivors in the random walk simulation was 0.11 mm/d higher than that of survivors in the constant growth rate run, and 90% of the survivors had growth rates above the original mean.

Discussion

The results of our simulations clearly demonstrate that both mean growth rate and growth rate variation among individuals can interact strongly with size-dependent mortality to cause significant effects on the number, growth rates, and final sizes of survivors. Consideration of variability among individuals may reveal insights regarding the processes shaping year class strength that are not available from analyses of population parameters and the dynamics of the average individual in a cohort. Our results are consistent with Sharp's (1987) suggestion that the characteristics of a small fraction of individuals in the cohort may largely govern recruitment success. Our simulations suggest that cohorts starting with similar mean growth rates but different variances may produce very dissimilar numbers and sizes of survivors, even when exposed to identical mortality mechanisms. Likewise, cohorts with very different initial mean growth rates can end up with very similar mean growth rates among survivors, if the variance in growth rates among individuals is different. For example, after 60 d the survivors from a cohort having an initial mean growth rate of 0.4 mm/d and low variance (upper center panel of Fig. 1) had a mean growth rate of 0.41 mm/d (SD = 0.13). After 60 d the mean growth rate of survivors from a cohort having a low initial mean growth rate of 0.2 mm/d and high variance (lower left panel of Fig. 1) had risen sharply to a similar value of 0.37 mm/d (SD = 0.12), not significantly different from the cohort with the high initial growth rate and low variance (based on 30 simulations; $P = 0.05$, $t = 1.906$, 171 df).

Our simulations also suggest that even though the vast majority of the mortality experienced by a cohort of larval fish occurs shortly after hatching when larvae are most numerous and may also be most vulnerable, in some cases selective mortality such as size-dependent predation after this period may substantially alter the number of larvae that survive. In our simulations, the distribution of survival probabilities among individuals changed, with time, from uniform to very nonuniform. Thus, chance events such as weather phenomena, variable transport dynamics, or other abiotic influences may govern mortality for the majority of a cohort, but size and growth rate characteristics of individuals, through their interaction with size-specific mortality, may still influence final cohort abundance and size structure. For example, after 15 d, our simulated cohorts with high and low growth rate variance and initial growth rate of 0.2 mm/d both showed very similar substantial mortality (survival = 11.5 and 11.1%, respectively), but after 60 d, survival in the high-variance cohort was nearly fourfold higher than in the low-variance simulation (1.1 versus 0.3%). In all our simulations, survivors tended to be fast-growing individuals, even though the vast majority of fast-growing fish still died. At the outset of our simulations, we could not predict which individuals would survive, but as growth and mortality proceeded, the outcome became increasingly clear. These results suggest that in some cases, year class strength may be set somewhat later in the early life history than has been commonly assumed (Sale 1990).

The patterns in survival, and in size and growth rate distributions, that we have simulated when keeping individual growth rates constant are conservative relative to the more realistic probability that individual growth rates will vary over time. The random walk trials we performed, which simulate this variability, suggest that when individual daily growth rates vary, the contribution of atypical individuals is further accentuated.

In the field, all mortality may not be size dependent. However, selection for fast-growing individuals may still persist. If we substitute random mortality for a portion of the size-dependent mortality, this has the same effect on the growth rate distribution as reducing the probability of encounter and attack by an equivalent proportion. We did not explicitly simulate the effects of size-dependent variation in growth rates, but such effects would also act to magnify the effects of individual variability.

The dynamics we describe emerged from simulations of small cohorts (typically 4000 individuals) with survival rates that are high relative to estimates for cohorts in the field (Forney 1976;

Ware and Lambert 1985; Houde 1987; Taggart and Leggett 1987). Results from the analytical model (Appendix) confirmed that much stronger patterns of selectivity for fast-growing individuals occur when cohort size is larger and more realistic mortality rates are used. This is so because selectivity increases with the amount of size-dependent mortality that occurs.

The results of our simulations suggest that progress toward an understanding of recruitment processes may be accelerated by a systematic and in-depth consideration of individual variability and the attributes of survivors. The specific results of our simulations, as with any model, derived from our assumptions. Our simulations were based on two general phenomena that are observed in many fishes: variability in growth rates and size-dependent vulnerability to predation. They demonstrate the potential for individual variability to substantially affect cohort survival and size structure of the survivors. Simulations exploring different assumptions (e.g. growing predators, different initial sizes, dome-shaped vulnerability functions, etc.) may provide additional insights, and should be pursued.

Individual-based Monte Carlo simulation models like the one presented here may also be useful in helping to determine the limits on our ability to predict cohort survival because they incorporate the uncertainty inherent in processes determining survival. For example, in the simulations we repeated 30 times (Table 1), the total range of survival values was two- to threefold. These results suggest an upper bound on the ability to accurately predict year-to-year variations in survival, even if we possessed models which perfectly summarized the recruitment process.

DeAngelis et al. (1993) compared the output of individual-based models like the one presented here with continuous models based on partial differential equations to evaluate the robustness of the simulation approach. In a variety of scenarios where both simulation and PDE models were applicable, both methods gave similar results, suggesting that either approach could be used. However, when growth rates are correlated among individuals (e.g. density or size dependence of growth; Lomnicki 1988) or when growth rates are correlated within individuals from day to day (our "random walk" with memory), it is difficult to solve the appropriate PDE model. In these and other biologically interesting situations (including those involving nonlinearities), the simulation approach used here may provide greater insights.

Individual-based simulation models are heuristically attractive and can be developed relatively easily even for situations where analytical solutions would be difficult or impossible to formulate and solve. However, individual differences may not be equally important in all stages of the life history. In our simulations, for example, individual differences in size become unimportant after all individuals have outgrown vulnerability to the predator. Individual-based models may be helpful in defining when a simpler approach to understanding year class variation, based on population averages, may be sufficient and when effects of individual variability in characteristics important to survival, such as body size, need to be considered.

Acknowledgments

This research was sponsored by the following agencies: Electric Power Research Institute under contract No. RP2932-2 (DOE No. ERD-87-672) with the U.S. Department of Energy, under contract No. DE-AC05-840R21400 with Martin Marietta Systems, Inc.; University of Wisconsin Sea Grant College Program and the state of Wisconsin through federal grant NA800AA-D-00086, Project R/LR-37; University of North Carolina Sea Grant College Program and the state of North Carolina through federal grant NA85AA-D-SG022, Project R/MER-12; NOAA South Atlantic Bight Recruitment Experiment (NA16RG0492-01), Project R/SAB-4; and the North Carolina Agricultural Research Station. T.J.M. and E.A.M. were supported in part by EPRI Fellowships in Population Biology administered through the Sports Fishing Institute. W. C. Leggett provided valuable comments on an earlier version of this paper. Publication No. 3971 of the Environmental Sciences Division, Oak Ridge National Laboratory.

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Appendix

The sytem under consideration is a fish cohort of individuals having different growth rates and suffering predation mortality with a probability that decreases with fish length. For cases in which the cohort can be described by a continuous distribution and in which the growth rates of individual fish do not vary randomly from day to day, it is possible to describe the changes in the size distribution of fish through time by means of a partial differential equation called the Sinko–Streifer model (Sinko and Streifer 1967). The model describes a system comparable with that described by the initial set of computer simulations in this paper. Whereas each simulation describes one specific possible outcome in this system, the Sinko–Streiffer model describes the long-term expectation. A second type of mortality, independent of size, can also be included or can be set to zero to correspond with simulations in the text.

Before formulating and solving this partial differential equation, let us define the relevant variables: N_0 = initial number of hatching fish, s = fish length, $F(s,t)$ = fish length frequency distribution at time t, s_0 = initial length of fish (at hatching), v = mean growth rate of fish (millimetres per day), $F_v(V)$ = distribution of growth rates, v, in the population

$$= N_0 \cdot \frac{1}{b \cdot \sqrt{2\pi}} \cdot e^{-\frac{1}{2}\frac{(v-\bar{v})^2}{b^2}},$$

Θ = individual probability of encounter and attack by a predator (per day), probability of capture given attack = $(13.5/s - 0.33)$ $(s_0 < s < s_1)$ or = 0 $(s_1 < s)$ (based on the size-dependent capture success relationship from Luecke et al. 1990), s_1 = length above which size-dependent mortality (predation) does not occur, and d_n = size-independent mortality rate coefficent (to correspond to simulations in text, $d_n = 0$). Each fish in the population can be thought of as having a growth rate, v, chosen initially from the distribution $F_v(V)$. Each individual's growth rate remains constant over time.

The dynamics of the length frequency distribution of the cohort through time can be described by the partial differential equation

$$\frac{\partial f}{\partial t} + v\frac{\partial f}{\partial s} = -\theta\left(\frac{13.5}{s} - 0.33\right)\cdot f - d_n \cdot f.$$

The solution of this equation for one single fish with growth rate v is

$$f(s,t;v) = \delta(s - s_0 - v\cdot t)\cdot \frac{e^{(0.33\cdot\theta - d_n)\cdot t}}{\left(1 - \frac{v\cdot t}{s_0}\right)^{\frac{13.5\cdot\theta}{v}}}$$

where $\delta(s - s_0 - v\cdot t)$ is the Dirac-delta function (see DeAngelis and Coutant 1979; DeAngelis and Mattice 1979; DeAngelis and Huston 1987).

To obtain $f(s,t)$, the size distribution for the whole population at time t, one must integrate $f(s,t;v)$ multiplied by the growth rate distribution, $F_v(V)$, over all values of v. In the present model all growth rates are constrained to the range (0,1) by setting $v = 0$ for all values of $V < 0$ in $F_v(v)$ and setting $v = 1$ for all values of $v > 1$ in $F_v(V)$. Then the integration yields

$$f(s,t) = f_1(s,t) + f_2(s,t) + f_3(s,t)$$

$$f_1(s,t) = F_1 \cdot e^{-\left(\left(\frac{13.5}{s_0} - 0.33\right)\cdot\theta + d_n\right)\cdot t} \quad \text{(for } s = s_0 \text{ only)}$$

$$f_2(s,t) = \frac{1}{b\cdot t\sqrt{2\pi}} \cdot e^{-\frac{(s - s_0 - \bar{v}t)^2}{2b^2t^2}} \cdot \left(\frac{s_0}{s_1}\right)^{\frac{13.5\cdot\theta\cdot t}{s - s_0}} \cdot e^{(0.33\cdot\theta - d_n)t}$$

(for $s < s_1$, $s < s_0 + t$)

$$f_2(s,t) = \frac{1}{b\cdot t\sqrt{2\pi}} \cdot e^{-\frac{(s - s_0 - \bar{v}t)^2}{2b^2t^2}} \cdot \left(\frac{s_0}{s_1}\right)^{\frac{13.5\cdot\theta\cdot t}{s - s_0}} \cdot e^{\left(\frac{0.33\cdot\theta(s_1 - s_0)}{s - s_0} - d_n\right)t}$$

(for $s > s_1$, $s < s_0 + t$)

$$f_3(s,t) = F_3\left(\frac{s_0}{s}\right)^{13.5\cdot\theta} \cdot e^{(0.33\cdot\theta - d_n)\cdot(s - s_0)}$$

(where $s = s_0 + t$ and for $s < s_1$)

$$f_3(s,t) = F_3 \left(\frac{s_0}{s_1}\right)^{13.5\cdot\theta} \cdot e^{(0.33\cdot\theta - d_n)\cdot(s_1 - s_0)}$$

(where $s = s_0 + t$ and for $s > s_1$)

where

$$F_1 = \int_{-\infty}^{0} F_v(v)\, dv$$

and

$$F_3 = \int_{1}^{\infty} F_v(v)\, dv\,.$$

Because all fish are the same size at $t = 0$, $f(s,t)$ can easily be converted to the distribution of growth rates in the cohort at time t.

Sediment pH and Redox Potential Affect the Bioavailability of Al, Cu, Fe, Mn, and Zn to Rooted Aquatic Macrophytes[1]

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Jackson, L. J., J. Kalff, and J. B. Rasmussen. 1993. Sediment pH and redox potential affect the bioavailability of Al, Cu, Fe, Mn, and Zn to rooted aquatic macrophytes. Can. J. Fish. Aquat. Sci. 50: 143–148.

We have evaluated the role of sediment pH (4.7–7.1) and redox potential (88–305 mV) in determining the bioavailability of five metals to four species of isoetoid macrophytes. The four species (*Eleocharis acicularis, Eriocaulon septangulare* With., *Isoetes* sp., and *Sagittaria graminae*) did not differ significantly in the relationship between the contents of metals in plants and sediment. When data for all species were pooled, 28–80% of the variation in the log of plant metal content was explained by the log of sediment metal (Al, Fe, and Mn) or by the log of sediment organic content (Cu and Zn). Sediment pH explained 45% (Al), 10% (Cu), 20% (Fe), 5% (Mn), and 12% (Zn) variation over and above that explained by the sediment metal content. These results demonstrate that lower sediment pH, in the presence of mildly oxic redox conditions, increases the bioavailability of these five trace metals to rooted aquatic plants. Rooted macrophytes subject to acidification contain higher metal concentrations which may be transferred by grazing to higher trophic levels.

Nous avons examiné dans quelle mesure le pH (4,7–7,1) et le potentiel redox (88–305 mV) des sédiments agissent sur la biodisponibilité de cinq métaux chez quatre espèces de plantes macrophytes isoétoïdes. Les quatre espèces (*Eleocharis acicularis, Eriocaulon septangulare* With., *Isoetes* sp. et *Sagittaria graminae*) ne différaient pas beaucoup au regard de la concentration des métaux dans les tissus et dans les sédiments. Lorsque les données de toutes les espèces sont réunies, il apparaît que 28 à 80 % de la variation du log de la teneur en métaux des plantes est expliqué par le log de la teneur des métaux dans les sédiments (dans le cas de l'Al, du Fe et du Mn) ou par le log de la teneur en matière organique des sédiments (dans le cas du Cu et du Zn). Le pH des sédiments permet d'expliquer 45 % (Al), 10 % (Cu), 20 % (Fe), 5 % (Mn) et 12 % (Zn) de la variation en plus du pourcentage expliqué par la teneur en métaux des sédiments. Ce résultat montre qu'un bas pH dans les sédiments, dans des conditions redox légèrement oxiques, accroît la biodisponibilité de ces cinq métaux à l'état de traces pour les plantes vasculaires aquatiques. Les macrophytes vasculaires exposés à l'acidification contiennent des métaux en concentration supérieure et ceux-ci peuvent être transférés aux niveaux trophiques supérieurs par broutage.

Received February 28, 1992
Accepted July 8, 1992
(JB420)

Reçu le 28 février 1992
Accepté le 8 juillet 1992

Metals from natural and anthropogenic sources exist in freshwater systems primarily adsorbed to fine-grained sediments (Gibbs 1973). Subsurface peaks in metal concentration typically occur in the top 10 cm of the sediment profile corresponding to a good portion of the rooting zone of many aquatic macrophytes. These subsurface peaks have been attributed to a variety of processes including an increase in atmospheric metal deposition (e.g. Ag, Au, Cd, Cr, Cu, Pb, Sn, V, and Zn; Galloway and Likens 1979), a decrease in watershed pH with a concomitant increase in metal mobility (e.g. Cronan and Shoefield 1979), and diffusion and/or precipitation along sediment redox gradients (Cu, Ni, Zn, Fe, and Mn; Carignan and Nriagu 1985). Surface littoral sediments therefore represent a potential concentrated buried reserve of trace metals that may be exported from the sediment or recycled by rooted aquatic macrophytes.

It is generally accepted that not all forms of a trace metal are equally available for uptake by biota (Luoma and Jenne 1976). A portion of the total metal in sediments may not be readily available to plants (e.g. adsorbed on particle surfaces, carbonate bound, occluded in iron and/or manganese oxyhydroxides, bound to organic matter, or sulfide bound); however, small changes in the sediment physicochemical environment may increase metal bioavailability. Nevertheless, Jackson et al. (1991) demonstrated that the aboveground element concentration of 39 elements in a variety of rooted aquatic macrophytes was closely related to the element's total concentration in the underlying sediment. They found a negative correlation between plant element concentration and both sediment Fe and organic content, supporting the hypothesis that these sediment constituents decrease element bioavailability. Sediment pH (range 6.2–8.3) did not explain residual variation in the plant–sediment element models probably because pH was restricted to a circumneutral range where the effect on element speciation, and thus bioavailability, was small. Because sediment pH and redox potential are two physicochemical properties known to regulate the chemical forms of heavy metals (Gambrell et al. 1976), variation in such sediment properties should result in variation in metal bioavailability.

Few studies have considered the role of pH in determining metal bioavailability to aquatic plants. Sprenger and McIntosh (1989) found that metal levels of *Utricularia purpurea* (no roots) were related more to water column pH than to the total metal. However, they did not consider sediment pH as a variable to explain rooted plant metal concentrations. A similar pH effect was noted by Lehtonen (1989) who found water column pH to be related to metal levels in *Nuphar lutea*, which obtains

[1]Contribution No. 317 to the Limnology Research Centre, McGill University.

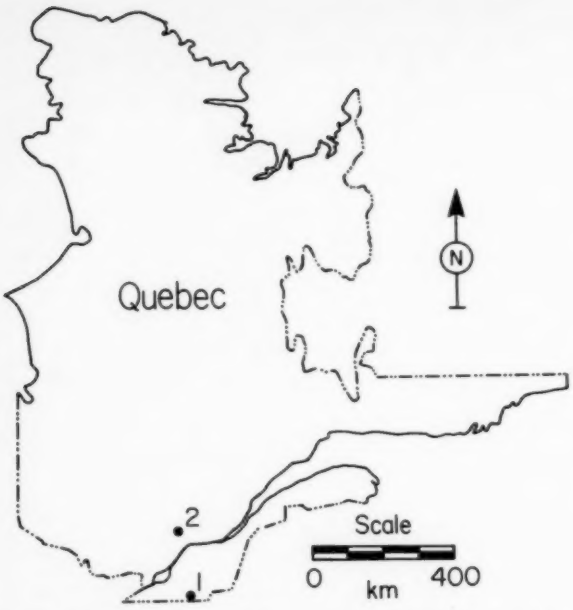

FIG. 1. Location of (1) Lake Memphremagog and (2) the Faunique Reserve Mastigouche in Quebec, Canada

most of its nutrition through leaves, while *Phragmites australis*, which obtains its nutrients primarily from the sediment, showed little response to water column pH in its metal accumulation. Gambrell et al. (1976) showed in laboratory studies that pH and redox potential differences affected the accumulation of Pb, Cd, and Hg in marsh plants but the degree to which their results are applicable to the field is unknown.

The purpose of this study is to test the hypothesis that low sediment pH results in an increase in the bioavailability of sediment metals and their content in rooted aquatic plants. We also consider the role of the redox potential in metal uptake by the plants.

Materials and Methods

Study Sites and Sampling Protocol

During August 1990, we sampled Lake Memphremagog (45°00′N, 72°10′W) in the Eastern Townships of Quebec and 12 lakes in the Faunique Reserve Mastigouche (46°40′N, 73°30′W), approximately 150 km northeast of Montreal, Quebec (Fig. 1). The three Lake Memphremagog sites were in the mesotrophic (chlorophyll *a* ~15 μg/L) south basin. The lakes in Mastigouche Park are typical small Canadian shield softwater lakes varying from clear to moderately coloured. Morphometric parameters are detailed in Rowan (1992).

At each site, whole plants were collected within a 30 × 30 cm quadrat by SCUBA divers. Quadrat positions were chosen to include each species present at that site. Plants were identified following Fassett (1957). On shore, shoots and roots were separated, shoots were washed and spin-dried in a salad spinner, fresh weight was determined with an electronic balance, and the shoot material was then stored on ice in ziplock freezer bags for up to 48 h prior to freezing. Roots were not included in the analyses.

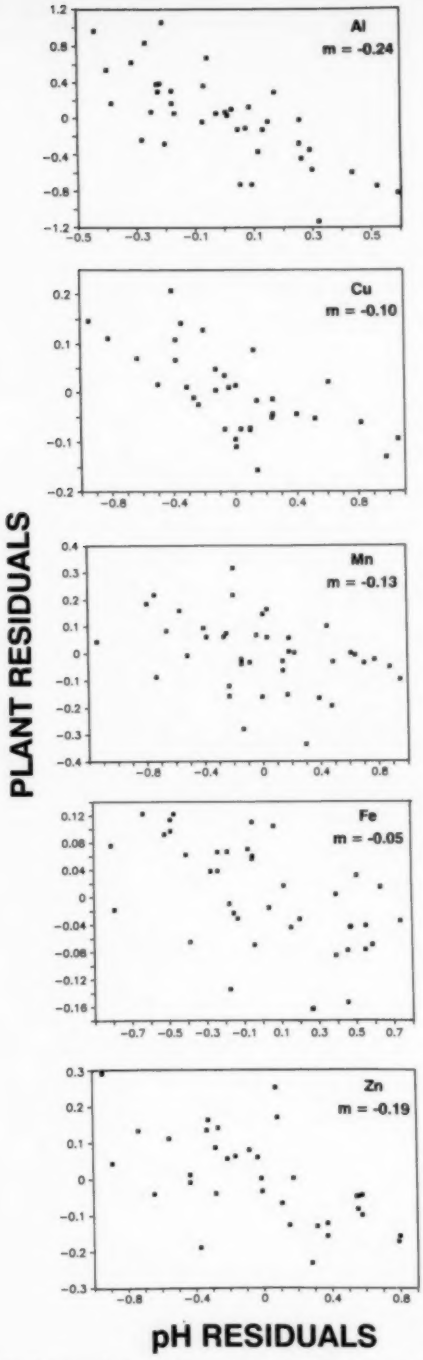

FIG. 2. Partial correlations between the log of plant metal and sediment pH for isoetoid sediments in Mastigouche Park, Quebec.

Immediately adjacent to the plant quadrat, five replicate sediment cores (to approximately 10 cm) were taken with Plexiglas core tubes (6.5-cm internal diameter). On shore, redox potential and pH (two and three replicate cores, respectively) were determined with a portable meter (Hach One pH meter model 43800-00) equipped with a flow-through pH

probe (model 44200-21) or a platinum redox electrode (model 44480-21) by inserting the appropriate probe into the top few centimetres of the sediment cores corresponding to the rooting depth of the plants. Meter calibration was performed at each site. After redox and pH had been determined, cores were extruded to plant rooting depth. Each extruded sample was then homogenized and a 15-mL subsample was placed in a scintillation vial, frozen for up to 2 mo, and then analysed for Al, Cu, Fe, Mn, and Zn. An additional subsample was dried to constant weight at 65°C and then burned at 550°C to estimate organic content as the grams lost on ignition as a percentage of the dry weight.

Metal Analyses

All labware was acid washed (10% HCl) and rinsed a minimum three times in double-distilled deionized water. All acids used were Analar grade (BDH Inc.). Dried sediments (1–3 g) were digested with dilute aqua regia (3:3:1 H_2O–HCl–HNO_3), while dried plants (1–1.6 g) were digested with concentrated HNO_3. Sediments and plants were digested with an acid to dry weight ratio of 15 mL : 1 g in 50-mL teflon tubes at room temperature overnight (~20°C), heated to 90 ± 5°C for 2 h, and allowed to cool. Cooled extracts were brought to 50 mL final volume in polyethylene volumetric flasks, spun at 2800 rpm for 10 min to remove suspended solids, and then analysed for Al, Cu, Fe, Mn, and Zn by flame atomic absorption spectroscopy (Perkin Elmer model 5100) with deuterium background correction for Fe. To assess extraction efficiencies, methodological variation between dates, and possible contamination, we extracted Buffalo River sediment (NBS ref. material 2704) and citrus leaves (NBS ref. material 1572) as reference sediment and plant materials. Concentrated HNO_3 extracted all plant metals with 100% efficiency. Dilute aqua regia extracted sediment Cu, Mn, and Zn with 100% efficiency, while Al and Fe were extracted with 18 and 52% efficiency, respectively. A dilute aqua regia digestion is therefore a partial metal digestion which undoubtedly selects a more bioavailable metal fraction from the sediments than would be the case with a total metal digestion because elements tightly bound within mineral structures would not be available to the plants over the time scale of growth.

Statistical Analyses

Multiple regression analyses fit plant element concentrations to linear and polynomial functions of sediment variables using the least-squares criterion. Because the nutrition of rooted aquatic plants has been experimentally shown to be largely sediment derived (Carignan and Kalff 1979, 1980; Barko and Smart 1981), we first examined the effect of sediment metal content before considering other sediment parameters. We then added pH and redox potential to the models to assess their contributions as additional model terms. To help demonstrate that our correlations are not spurious, we examined graphically the partial correlations between the log of plant metal content and pH by plotting the residuals of the log plant metal term (with pH removed) against the residuals of pH regressed on the other independent variables (Fig. 2). Variables were logarithmically transformed, when necessary, to remove heteroskedastic variation (Sokal and Rohlf 1981).

Results

Study Sites and Their Sediments

Most sediments had redox potentials between 80 and 200 mV. A few sites were above 200 mV, but none fell below 80 mV (raw data published in Jackson 1992). There was a tendency for the highest redox potentials to come from sites with the highest exposures (Lakes Loutre, Memphremagog, and St. Bernard). The traditional anaerobic/aerobic transition zone occurs at 200 mV for soils (Ross 1989) and sediments (Berner 1981). Our sediments therefore include sites that are mildly reducing to moderately oxic and this is consistent with the fact that isoetoid macrophytes can oxygenate their substrata (Wium-Andersen and Andersen 1972; Tessenow and Baynes 1975, 1978; Sand-Jensen et al. 1982; Jaynes and Carpenter 1986). The redox potentials we found are slightly lower than those previously reported for isoetoid-bearing sediments (~300–600 mV; Tessenow and Baynes 1978) but above the typical range for nonisoetoid, vegetated sediments (100 to −300 mV; Gambrell et al. 1976).

Sediment pH spanned over three orders of magnitude from 4.8 to 7.1. This range is much larger than that found in a literature survey of lakes and rivers where the lowest value was 6.2 (Jackson et al. 1991). Using experimental Plexiglas chambers, Tessenow and Baynes (1978) found that an increase in sediment redox potential was accompanied by a decrease in sediment pH to as low as 4.8 within the rooting zone of *Isoetes lacustris*. However, our field observations did not show a significant correlation between sediment E_h and pH (Table 1). No sediment parameter was significantly correlated with pH.

Sediment Mn and Fe were significantly correlated with each other and both metals were significantly correlated with redox potential. These findings are not surprising given the similar behavior of Mn and Fe and Fe's strong influence in setting the redox potential in freshwater sediments. The log of sediment organic content was weakly correlated with the log of sediment Cu and Zn. Al, Cu, Fe, and Mn were significantly intercorrelated with one exception (Al and Mn).

Plant–Sediment Metal Relationships

The relationship between plant and sediment metal content for Al, Fe, and Mn was best described by a parabolic function (e.g. Mn; Fig. 3A) which explained 28–80% of the variation in the log of plant metal. Although the highest sediment metal values come from the highly oxic Lake St. Bernard sediments, the removal of Lake St. Bernard from the data set fails to linearize the relationships, and therefore, these parabolic models are not driven by one lake alone. Moreover, the linear models for Cu and Zn, two elements less strongly influenced by the redox potential, are not biased by including Lake St. Bernard. In contrast with Al, Fe, and Mn, the log of sediment organic content explained the largest amount of variation in the plant Cu and Zn content. Sediment Cu and Zn were not significant terms in their respective models. Plant Cu and Zn contents were related to linear functions of sediment organic content (e.g. Cu; Fig. 3B), and the positive coefficient of log organic content indicates that increases in sediment organic matter was correlated with increases in plant Cu and Zn contents.

The negative pH coefficients in all models (Table 2) show that plants from low-pH sediments have higher metal content than would be expected from the sediment metal content. This result is likely restricted to sediments of low to neutral pH (4.8–7.1 in this study), as a pH range of 6.2–8.3 failed to explain

TABLE 1. Matrix of simple correlation coefficients for variables in isoetoid-bearing sediments of Mastigouche Park, Quebec. Metal and organic contents (org; grams lost on ignition at 550°C) were log transformed. *Significant correlation ($p < 0.05$) as determined with a Bonferroni adjusted probability.

	org	pH	redox	Al	Fe	Mn	Cu	Zn
org	1.00							
pH	−0.22	1.00						
redox	−0.41	0.13	1.00					
Al	0.28	−0.30	0.27	1.00				
Fe	−0.18	−0.03	*0.65	*0.61	1.00			
Mn	−0.09	−0.35	*0.66	0.36	*0.76	1.00		
Cu	0.49	−0.35	0.32	*0.64	*0.67	*0.63	1.00	
Zn	0.47	−0.17	0.23	0.30	0.32	0.37	0.51	1.00

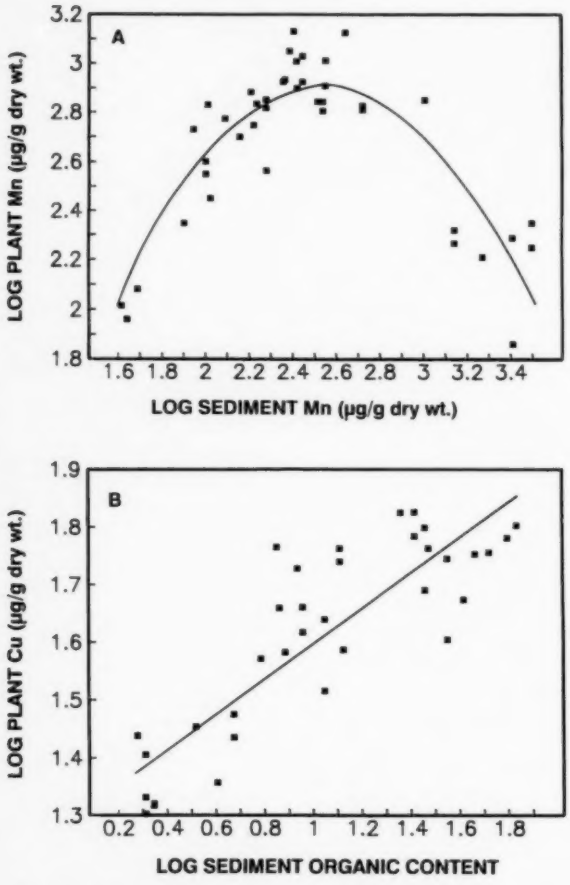

FIG. 3. Relationship between (A) Mn and (B) Cu in isoetoid macrophytes and their underlying sediments for 13 lakes in Quebec, Canada, during August 1990.

any of the variation in plant metal burdens for the same metals in a previous study (Jackson et al. 1991). The present findings are consistent with previous studies (Gambrell et al. 1976; Carignan and Nriagu 1985) that show a general pattern of increasing metal mobility with decreasing pH < 6.5.

The pH term explained 5% (Mn) to 45% (Al) of the variation in the log of plant metal content over and above that explained by the sediment metal terms. The residuals of the partial correlations between the log of plant metal and pH are evenly distributed (Fig. 2). Therefore, the results for pH do not simply reflect the influence of a few heavily weighted data. There was a weak correlation between pH and sediment organic content ($r = 0.22$).

TABLE 2. Regression models obtained in relating the aboveground plant metal content (µg/g dry weight) to sediment properties for five trace-metals in Quebec lakes supporting isoetoid macrophytes. Al, Fe, and Mn are expressed as µg/g dry weight. Sediment organic content (ORG) is the grams lost on ignition at 550°C divided by the dry weight of the sediment. The standard error of the model term is indicated in parentheses. P and S represent plant and sediment, respectively.

Al: log P(Al) = −21.08(5.10) + 12.96(2.71) log S(Al)
−1.67(0.36) log S(Al)2
−0.24(0.03) pH
$r^2 = 0.73$ $SE_{est} = 0.16$

Cu: log P(Cu) = 1.95(0.04) + 0.23(0.03) log ORG
−0.10(0.03) pH
$r^2 = 0.85$ $SE_{est} = 0.07$

Fe: log P(Fe) = −5.91(1.03) + 4.58(0.51) log S(Fe)
−0.56(0.06) log S(Fe)2
−0.05(0.01) pH
$r^2 = 0.85$ $SE_{est} = 0.04$

Mn: log P(Mn) = −2.59(0.62) + 4.91(0.41) log S(Mn)
−0.97(0.08) log S(Mn)2
−0.13(0.04) pH
$r^2 = 0.85$ $SE_{est} = 0.14$

Zn: log P(Zn) = 2.66(0.26) + 0.32(0.05) log ORG
−0.19(0.04) pH
$r^2 = 0.85$ $SE_{est} = 0.12$

Discussion

Because plants take up elements in ionic form (Anderson 1975; Fitter and Hay 1983), it should be possible to predict sediment metal bioavailability to rooted aquatic plants if accurate partitioning of the total metal between ionic and nonionic forms within the sediment is known. While the literature contains pH – redox potential diagrams outlining partitioning processes in inorganic aqueous solutions (e.g. Hem 1972; Stumm and Morgan 1981), their use in calculating accurate partitioning in lake sediments is problematic because these methods assume simple inorganic systems in thermodynamic equilibrium. Lake sediments will be much more diverse in both the quantity and quality of reactive constituents. One approach to examine the role of pH and redox potential in regulating the bioavailability of metals to aquatic macrophytes is to use representative sediments.

A quantitative interpretation of E_h in terms of its composition in freshwater sediments is not possible due to a number of fac-

tors that affect the measurement (Whitfield 1969). Not all redox couples react with E_h electrodes, and most redox systems are not in thermodynamic equilibrium. These factors do not, however, diminish the usefulness of E_h in characterizing sediments (Zobell 1946). The redox potential of freshwater littoral sediments has been shown to be strongly linked to environmental variables; for example, Rasmussen (1988) found that sediment redox potential was a positive function of site exposure and slope and a negative function of measures of lake trophic status such as macrophyte biomass and chlorophyll *a*. In our data, too, sites with the highest exposures (Lakes Memphremagog and St. Bernard) had the highest redox potentials. Sediment redox potential thus appears to be affected by the energy environment at the site but can be additionally modified on a local scale by the plant community present.

The sediment domain may be classified into three redox potential zones (Berner 1981). In strongly reduced sediments ($E_h < -150$ mV), S will exist primarily as sulfide and form insoluble metal sulfide precipitates (Engler and Patrick 1975). Within the intermediate zone between strongly reduced and strongly oxidized sediments ($-150 > E_h > +200$ mV) the greatest metal mobility probably occurs. In strongly oxidized sediments, S will exist primarily as SO_4^{2-} and Fe and Mn will occur primarily as insoluble hydrous oxides. It has been shown that hydrous oxide formation can be a significant trapping mechanism (from the water column and/or the mobile sulfate zone) for metals where such oxides are formed (Jenne 1968). Because sediments influenced by the rooting zone of macrophytes will not be in thermodynamic equilibrium, we cannot use E_h as any more than a rough indicator of the form in which an element will exist. However, that we were able to correlate levels of Fe and Mn with redox potentials indicates that high metal oxide levels were characteristic of our high-E_h sites, and thus, E_h appears to be a useful general descriptor of the chemical environment surrounding plant roots.

Our models for Al, Mn, and Fe contrast with a previous survey of 39 elements from reduced sediments where 75% of the plant element burden was explained by the total sediment element term with log-linear slopes of 1 (Jackson et al. 1991). It is important to note the differences between the sediments within these two studies. In the literature survey the sediment redox potential, when given, was -100 to -250 mV for non-isoetoid-bearing sediments compared with $+88$ to $+305$ mV for the present study. Given the high redox potentials of isoetoid sediments, it is likely that Al coprecipitates with or adsorbs to hydrous iron and manganese oxides in the plant's rooting zone, with the result that these metals reach high concentrations in the upper layers of the sediments but are at the same time largely unavailable to the plants for uptake. This scenario could lead to the parabolic patterns we observe in the plant – sediment metal relationships (Fig. 3A) and would also explain why parabolic plant – sediment metal relationships were not obtained in our previous study where E_h's were strongly reducing.

Because plant Cu and Zn contents were positive linear functions of sediment organic content, we hypothesize that in our sediments the available Cu and Zn was associated with the organic material in the sediment. This is consistent with the notion that Cu and Zn preferentially bind to organic ligands but inconsistent with the hypothesis that organic ligands make elements less available to biota (Campbell et al. 1988). It should be noted that the range of sediment Cu and Zn was only one order of magnitude or less. That sediment metal was not a significant model variable does not mean that the sediment metal is not functionally important. In a previous study (Jackson et al. 1991) where Cu and Zn ranged approximately two orders of magnitude, sediment Cu and Zn were significant models terms, just as sediment metal is here for Al, Fe, and Mn.

Because four species of macrophytes were present, it is possible that between-species differences might be an important source of variability in the models. We investigated this by analysis of covariance but found neither a significant species effect nor any interactions involving species and other predictors. While this does not prove that there is no difference in metal accumulation between the four macrophyte species, it does mean that we cannot discern any species effect that might exist. Models for *Eriocaulon septangulare*, the species for which we have the most data, are not significantly different from the combined models. Pooling the data for the four species reduces the precision of our models but increases their generality. The lack of a species effect is consistent with previous studies (Pip 1990; Sprenger and McIntosh 1989) that were unable to find patterns in metal concentration in rooted aquatic macrophytes.

As isoetoid macrophytes are perennial plants, it is impossible to factor plant age into the analysis. Because age has been shown to affect metal content in shoots of submerged plants (Mayes and McIntosh 1975; Mayes et al. 1977; Welsh and Denny 1980), variability in the age of the plants must contribute to the unexplained variation in the present study.

Our results indicate that low sediment pH and slightly positive redox potentials ($0 < E_h < 200$ mV) increase the bioavailability of the nonresidual fraction of the sediment metal pool. These results are most applicable to softwater lakes where sandy sediments have a poor capacity to buffer increased acid inputs and where the rooted aquatic plants are dominated by isoetoid macrophytes.

While we studied only five metals, our results should have relevance to other metals/elements because the physicochemical attributes of the elements have been shown to be related to elemental behaviour in biological systems (Kaiser 1980, 1985; Krantzberg 1989). We predict that plant element concentrations of those elements whose geochemical forms are affected by pH and redox potential should show similar patterns in their plant–sediment relationships. If so, our findings could be extended beyond the five metals we studied. Our findings point to the important role that rooted aquatic plants can play in the transfer of sediment-bound metals to higher trophic levels.

Acknowledgements

We thank G. Keeting for advice and help with the metal analyses, V. Guillmettre for providing a canoe, lodging, and access to the Mastigouche Park lakes, and J. Dion for help with the field sampling. This work was supported by a Natural Sciences and Engineering Research Council of Canada grant to J. Kalff and a Quebec Les Fonds Pour la Formation de Chercheurs et à l'Aide à la Recherche (FCAR) team grant to J. Kalff, R. Peters, and J. Rasmussen.

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Pelagic Food Web Structure in Adirondack Mountain, USA, Lakes of Varying Acidity

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Havens, K. E. 1993. Pelagic food web structure in Adirondack Mountain, USA, lakes of varying acidity. Can. J. Fish. Aquat. Sci. 50: 149–155.

Pelagic food web models were constructed for 50 softwater lakes of varying acidity (pH 4.17–7.32) in the Adirondack Mountains, and relationships between food web parameters and several abiotic variables (pH, monomeric Al, dissolved organic carbon (DOC), z_{max}, and area) were quantified. With declining pH, pelagic food web complexity was reduced, as the number of species, links, links per species, predator generalization, and prey vulnerability all declined. The declines were less pronounced in lakes having high DOC levels. Some web parameters, including the predator/prey ratio, the portion of basal, intermediate and top species, and species redundancy, did not decline with pH but were more variable among the acid lakes. Those trends are consistent with the view that at low pH, toxicity becomes the major factor controlling community structure.

On a créé des modèles de réseau trophique pélagique pour 50 lacs d'eau douce situés dans les monts Adirondacks et ayant divers degrés d'acidité (pH de 4,17 à 7,32), puis on a quantifié les liens entre les paramètres du réseau trophique et plusieurs variables non biotiques (le pH, l'Al monomère, le carbone organique dissous (COD), z_{max} et la zone en cause). La diminution de la complexité du réseau trophique est proportionnelle à la réduction du pH, puisque le nombre d'espèces, de maillons, de maillons par espèce de même que le nombre moyen d'espèces consommées par prédateur et la vulnérabilité des proies ont tous diminué. Les baisses sont moins prononcées dans les lacs présentant des concentrations élevées de COD. Certains paramètres du réseau trophique, notamment le rapport prédateurs/proies, la proportion d'espèces se situant à la base, au milieu et au sommet de la chaîne alimentaire ainsi que le rapport du nombre de taxons biologiques sur le nombre d'espèces trophiques n'ont pas diminué en présence d'un pH plus faible, mais ils varient davantage dans les lacs acides. Ces tendances étayent l'hypothèse selon laquelle en présence d'un pH faible, la toxicité constitue le facteur principal influant sur la structure des communautés.

Received January 14, 1992
Accepted July 9, 1992
(JB368)

Reçu le 14 janvier 1992
Accepté le 9 juillet 1992

Lake acidification greatly impacts the pelagic community, eliminating sensitive species at all trophic levels. Numerous studies have documented the responses of phytoplankton (e.g. Siegfried et al. 1989; Findlay and Kasian 1990; Pinel-Alloul et al. 1990b), zooplankton (e.g. Confer et al. 1983; Siegfried et al. 1984; Pinel-Alloul et al. 1990a; Tessier and Horwitz 1990), and fish (e.g. Mills et al. 1987; Matuszek and Beggs 1988; Henriksen et al. 1989) assemblages to declining pH. Multi-trophic level and whole-community responses have also been described (e.g. Schindler et al. 1985; Stenson 1985; Siegfried et al. 1987a, 1987b). However, no study has quantified acidification impacts on the overall structure of the pelagic food web. Because phytoplankton, zooplankton, and fish are members of that web, acidification may have both direct toxic effects and indirect effects due to altered food web interactions (Eriksson et al. 1980; Havens and DeCosta 1985; Olofsson et al. 1988). Hence, our understanding of the biotic responses to lake acidification could be enhanced by investigations in which the entire food web is the unit of study. Recently, I quantified the effects of acidification on zooplankton food web structure of 25 softwater lakes in the northeastern United States (Havens 1991). I found that with declining pH, there were significant reductions in several web parameters, including species richness, linkage density, predator generalization, and the model number of trophic levels. In contrast, both the degree of omnivory and cannibalism increased at lower pH. The trends indicate that acidification causes a decline in zooplankton food web complexity, and perhaps also a reduction in energy flow to higher trophic levels. However, it was unclear whether the entire pelagic web responds to acidification in this manner. The only other study to investigate relationships between acidity and pelagic food web structure included only the zooplankton component of the webs (Locke 1992).

In the present study, I examined the "complete" pelagic food webs (piscivores, planktivores, zooplankton, and phytoplankton) of 50 softwater lakes of varying pH (4.17–7.32) in the Adirondack Mountains of New York State and quantified relationships between food web parameters and several abiotic variables, especially pH. Although the webs described herein lack certain components (e.g. bacteria and ciliate protozoans), this paper provides the most complete taxonomic coverage of any food web study of acidified lakes to date.

Materials and Methods

Data Set

The physical and chemical data and the species lists used to construct the food webs were obtained from a technical report of the New York State Department of Environmental Conservation (Sutherland 1989). The report was the result of a 50-lake survey in the Adirondack ecological zone. The lakes were located at 479–811 m elevation, ranged in size from 2 to 182 ha, and had a maximum depth of 0.6–24.4 m, pH 4.17–

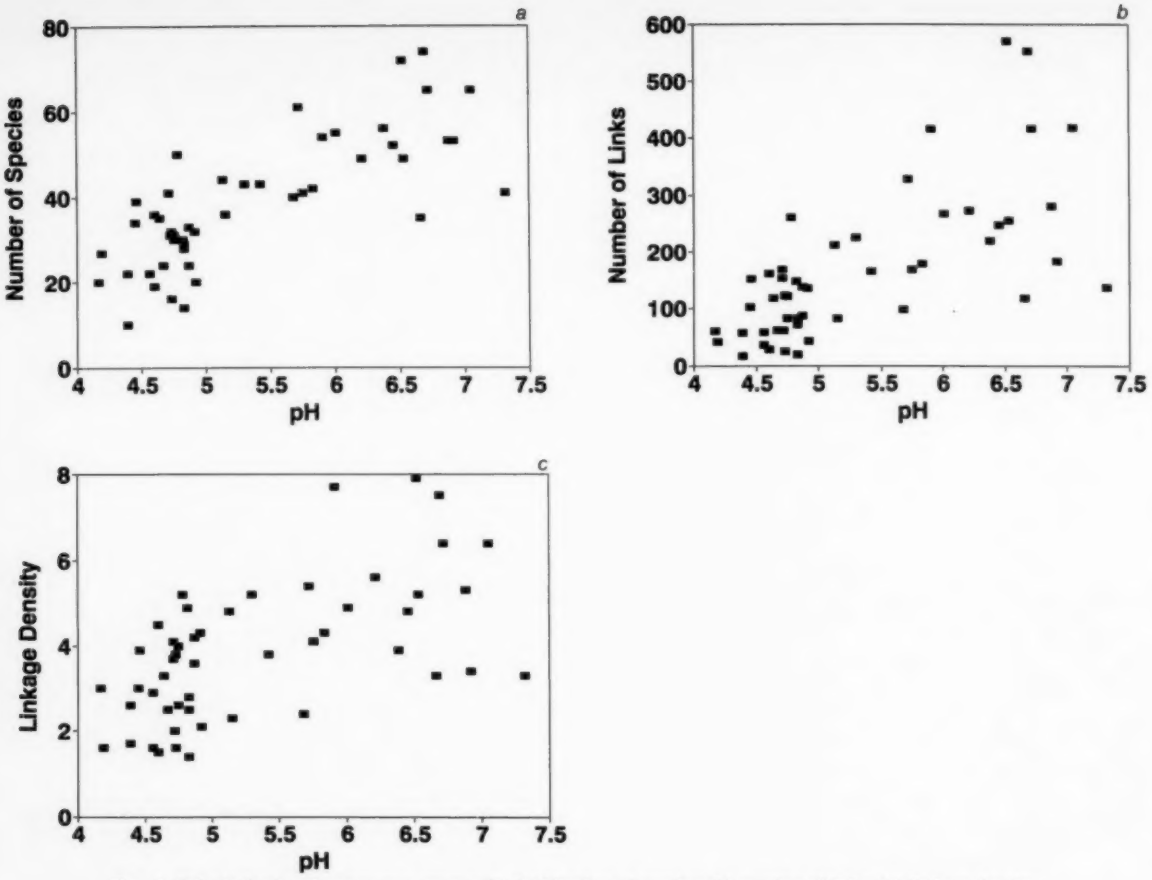

FIG. 1. Pelagic food web parameters versus pH. (a) Number of species; (b) number of links; (c) linkage density.

7.32, monomeric Al of 0–41 $\mu g \cdot L^{-1}$, dissolved organic carbon (DOC) of 1–14 $mg \cdot L^{-1}$, and total P of 5–41 $\mu g \cdot L^{-1}$. The lakes were selected by a stratified random design, described in detail in Sutherland (1989). All 50 lakes were sampled once during summer 1984 for chemistry, phytoplankton, zooplankton, and fish. Details of the methods used to collect and process samples are given in Bell et al. (1989), Quinn et al. (1989), Siegfried (1989a, 1989b), and Sutherland and Bloomfield (1989). Briefly, samples for chemical analyses and phytoplankton enumeration were taken from midepilimnion at the deepest site in each lake, and zooplankton were collected with replicated vertical tows of a plankton net (76 μm for rotifers, 158 μm for crustaceans). pH and other water chemistry variables were analyzed using standard U.S.E.P.A. methodology; phytoplankton were enumerated using the Utermöhl technique; zooplankton were enumerated in counting chambers. Fish were sampled using overnight gill nets and minnow traps. The single visit to each lake may not have resulted in complete species lists. Sprules and Bowerman (1988) showed that a single midsummer sampling can provide a "reasonable estimate of zooplankton food web structure" for softwater lakes. It is unclear whether this statement is valid for the phytoplankton. However, since my objective was to make interlake comparisons, the most important point is that a consistent methodology was used to sample the 50 food webs.

Food Web Construction

The first step in constructing the food webs was to produce a cumulative list of species collected in the 50 lakes. The term species is used here to describe biological taxa, rather than trophic species (Briand and Cohen 1984). Trophic species are groups of biological taxa which share common predators and prey. Although trophic species are the unit of study in most food web research (e.g. Briand 1983; Briand and Cohen 1984; Cohen and Briand 1984; Schoener 1989), biological taxa are used here because they are the more conventional unit in aquatic ecology research. Also, the major reason for lumping taxa into trophic species, to remove bias caused by greater taxonomic resolution at higher trophic levels (Pimm 1982), is not a problem here. Nearly all fish, zooplankton, and phytoplankton were resolved to the biological species level.

Some plankton were reported as unidentified but distinct species within a genus, e.g. "*Mallamonas species* A". In addition, I added developmental categories for species that undergo ontogenic changes in diet and/or susceptibility to predators. These additional lumped "trophic taxa" (Martinez 1991) were nauplii, fish fry, and fish eggs. The category detritus was also included as a food source for benthivorous fish and cladocerans. Information about the diet of each species was taken from the literature, especially Scott and Crossman (1973), Bogdan and Gilbert (1984), Havens and DeCosta (1985), Sprules and

TABLE 1. Results of the stepwise multiple regression analyses of Adirondack pelagic food web parameters in relation to selected abiotic variables. R^2 for entry into the model = 0.05. Only those parameters for which significant models could be constructed are listed. S = number of species, L = number of links, d = linkage density, G = generalization, V = vulnerability, DOC = dissolved organic carbon.

Parameter	Step	Variable	Partial R^2	Model R^2	F	$p > F$
S	1	pH	0.59	0.59	69.6	0.0001
	2	Area	0.08	0.67	12.4	0.001
L	1	pH	0.48	0.48	43.9	0.0001
	2	Area	0.14	0.62	16.5	0.0002
	3	DOC	0.07	0.69	10.0	0.003
d	1	pH	0.36	0.36	26.6	0.0001
	2	Area	0.10	0.46	9.5	0.004
	3	DOC	0.09	0.55	7.1	0.01
G	1	pH	0.43	0.43	35.6	0.0001
	2	Area	0.08	0.51	7.9	0.007
	3	DOC	0.07	0.58	7.9	0.007
V	1	pH	0.31	0.31	21.4	0.0001
	2	Area	0.10	0.41	8.6	0.005
	3	DOC	0.09	0.50	6.6	0.01

Bowerman (1988), and Martinez (1991), and used to construct a cumulative matrix of predator–prey interactions among the 220 taxa. The matrix consisted of rows representing prey and columns representing predators. Each a_{ij} element in the matrix was assigned a value of unity if the ith prey was preyed upon by the jth predator and a value of zero if not. Individual food web matrices were then constructed for the 50 lakes by extracting from the cumulative matrix those rows and columns corresponding to the species present in each lake. In some cases, the lake matrices were edited by deleting "dangling species" (Schoener 1989) which lacked trophic links to the web.

The disadvantage of this approach to constructing food webs is the assumption that interactions do not vary from lake to lake, i.e. if species A eats B in one community, it eats B in all other communities where both are present, regardless of the availability of alternative prey. This assumption is probably not valid, especially for fish. However, it is an assumption made in nearly all multiweb studies. To directly establish links, it would be necessary to perform gut content analyses of all pelagic predators; that approach would limit the study to only a small number of lakes.

Food Web Analyses

For each lake matrix, the following parameters of food web structure were determined:

(1) *Number of species*: the total number of biological taxa in the web.

(2) *Number of links*: the total number of predator–prey interactions, i.e. the sum of all a_{ij} elements in the matrix.

(3) *Linkage density*: the total number of links divided by the number of species.

(4) *Generalization*: the average number of prey exploited by each predator in the web (Schoener 1989).

(5) *Vulnerability*: the average number of predators exploiting each prey in the web (Schoener 1989).

(6) *Degree of omnivory*: the portion of predatory species in the web that prey on more than one trophic level.

(7) *Degree of cannibalism*: the portion of predatory species in the web that prey on members of their own species.

(8) *Portion of species in top, intermediate, and basal trophic categories*: the number of top, intermediate, and basal species divided by the total number of species in the web. Intermediate species have both predators and prey; basal species have predators only (Briand and Cohen 1984). I defined top species as having no predators except those involved in reciprocal predation (species A eats B and vice versa) or cannibalistic interactions. This eliminates a major problem with the traditional definition of a top species (having prey but no predators); it is unlikely that any such species actually exist in nature (Martinez 1991). For example, brook trout (*Salvelinus fontinalis*) are clearly the top predator in lakes containing trout, bluegill (*Lepomis macrochirus*), shiners, and plankton. Yet, large trout cannibalize smaller members of their own population. Similarly, brook and brown trout (*Salmo trutta*) may be the top predators in a lake, yet prey on each other's young as well as their own.

(9) *Predator/prey ratio*: the sum of top and intermediate species divided by the sum of intermediate and basal species (Cohen 1978).

(10) *Species redundancy*: the number of biological taxa divided by the number of trophic species.

Relationships between the food web parameters and several abiotic variables (pH, monomeric Al, DOC, total P, z_{max}, and area) were tested statistically using the correlation and stepwise multiple regression procedures of SAS Stat Version 5 (SAS Institute 1985) and the Kent State University IBM mainframe computer. The six abiotic variables were chosen because they were previously found to be correlated with fish and/or plankton community structure in these lakes (Siegfried et al. 1987a, 1987b, 1989).

Results and Discussion

The number of species (Fig. 1a) was positively correlated with pH ($r^2 = 0.59$, $p < 0.01$), reflecting the previously documented relationships between species number (S) and pH for the plankton (Siegfried et al. 1987a) and fish (Bell et al. 1989) of these lakes. Similar relationships have been reported for phytoplankton, zooplankton, and fish in numerous studies of acid-impacted North American lakes. However, the present study is the first to quantify the S versus pH relationship for a complete pelagic community.

S was also correlated with lake area ($r^2 = 0.31$, $p < 0.05$) and negatively correlated with monomeric Al concentration ($r^2 = 0.34$, $p < 0.05$), reflecting the increase in monomeric Al with declining pH (Quinn et al. 1989) in the study lakes. The best fit multiple regression model contained only pH and lake area as independent variables (Table 1) and accounted for 67% of the variation in S. The relationship between lake area and S has been previously demonstrated. For example, Rahel (1986) showed that small Wisconsin lakes supported fewer fish species than larger lakes, and Sprules (1975) found that lake size accounted for a significant portion of the variation in crustacean zooplankton S in central Ontario lakes.

There were significant positive relationships (Fig. 1b, 1c) between the total number of trophic links (L) and pH ($r^2 = 0.48$, $p < 0.01$) and between linkage density (d) and pH ($r^2 = 0.36$, $p < 0.05$). The latter relationship is consistent with that previously reported for zooplankton food webs (Havens 1991; Locke 1992) and indicates an overall decline in pelagic food web complexity with acidification.

The results do not support the hypothesis of Cohen and Briand (1984) and Cohen and Newman (1988) that d is invariant among food webs of varying S (the "link species scaling law"). Rather, the results support Schoener's (1989) and Martinez's (1991) view that d and S are positively related. As was the case with S, pH explained most of the variation in d and L (Table 1), and lake area was also a significant component of the multiple regression model. Furthermore, for both food web parameters, DOC entered the model as a third independent variable. Both L and d tended to be slightly higher in acid lakes having elevated DOC concentrations. This result is consistent with previous studies which showed that DOC ameliorates the toxic effects of Al on aquatic biota (McCahon and Pascoe 1989; Spry and Wiener 1991).

Generalization (Fig. 2a) also showed a significant positive correlation with pH ($r^2 = 0.43$, $p < 0.01$), as did vulnerability (Fig. 2b) ($r^2 = 0.31$, $p < 0.05$). In each case, the best fit regression model contained pH, lake area, and DOC as the significant independent variables (Table 1). The trends in generalization and vulnerability reflect in part those previously reported (Schoener 1989) between these parameters and S. Schoener found that for 17 lentic food webs, generalization increased by 1 and vulnerability increased by 2.5 when S increased from 5 to 30 species. The slopes of regression lines fitted to Schoener's data are 0.04 for generalization versus S and 0.10 for vulnerability versus S. In the present study, generalization increased roughly threefold and vulnerability increased roughly fourfold over a range of 10–72 species. The regression line slopes were 0.17 and 0.10, respectively. These

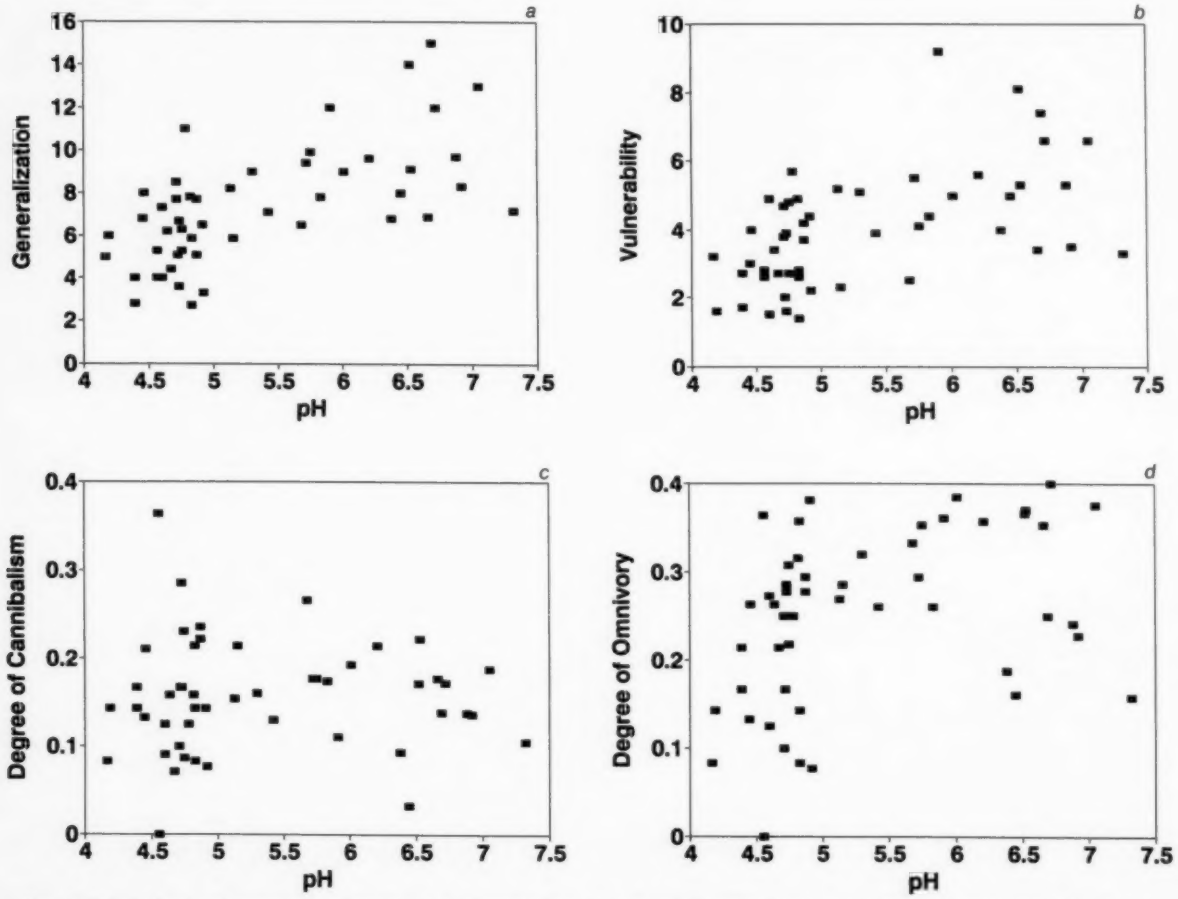

FIG. 2. Pelagic food web parameters versus pH. (a) Generalization; (b) vulnerability; (c) degree of cannibalism; (d) degree of omnivory.

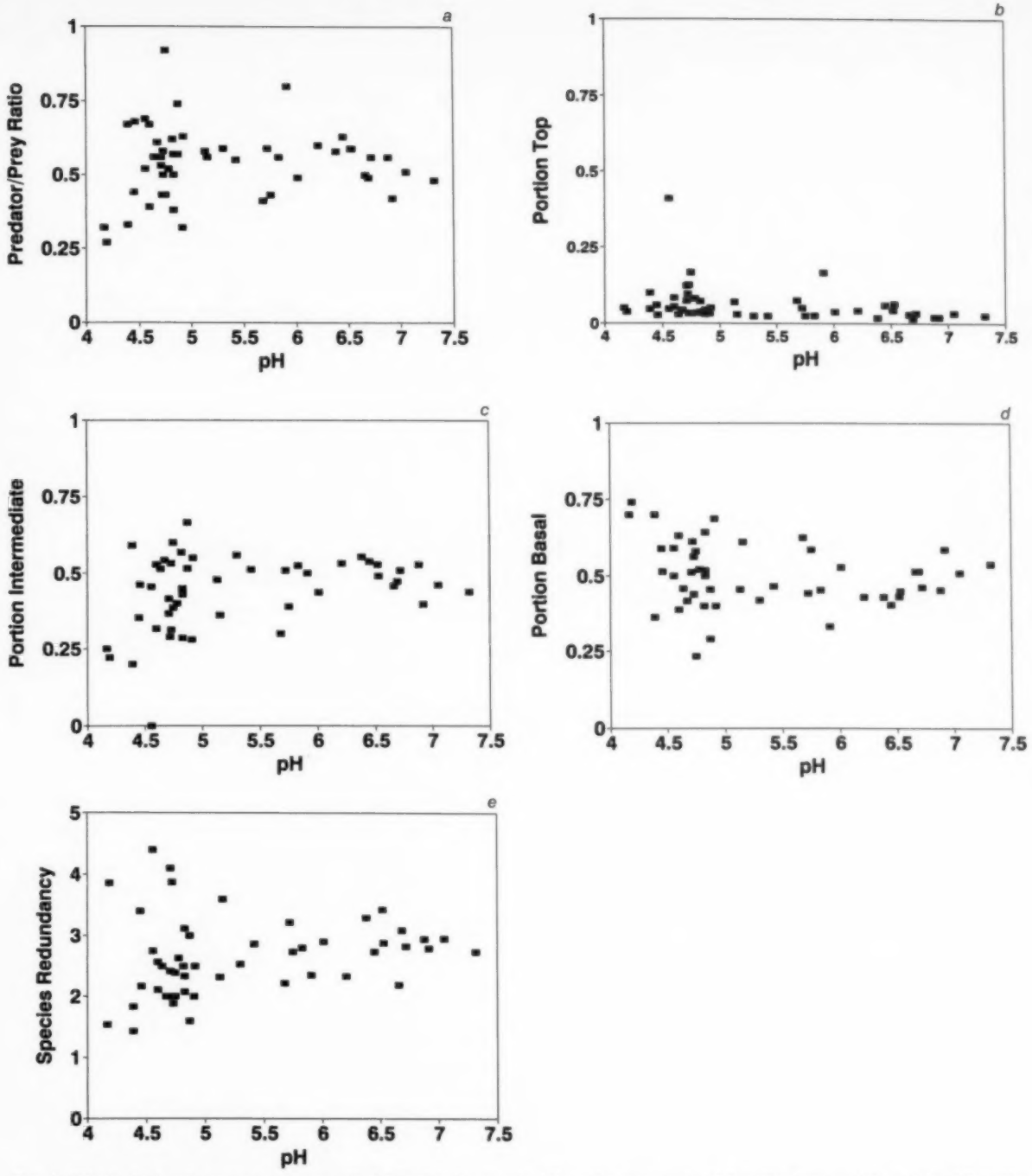

FIG. 3. Pelagic food web parameters versus pH. (a) Predator/prey ratio; (b) portion top species; (c) portion intermediate species; (d) portion basal species; (e) species redundancy.

results indicate that with acidification, there is a significant decline in the number of prey exploited by any given predator and also a decline in the number of predators exploiting any given prey. The former (generalization) is reduced to an extent which exceeds that expected based on differences in S alone. This finding is consistent with the previous result for zooplankton webs (Havens 1991).

In the study of zooplankton food webs (Havens 1991), I also found that the degree of omnivory and cannibalism increased with declining pH. Those trends were said to reflect the fact

that alternative feeding modes could allow populations to survive during periods of reduced energy flow from adjacent trophic levels. The net result would be an increase in food web stability, as suggested by Lane (1978) and Pimm (1982). Previous studies have also shown that omnivory and cannibalism are common in the pelagic food webs of unproductive lakes (Sprules and Bowerman 1988; Martinez 1991), as they were in the present study (Fig. 2c, 2d). However, I found no significant relationship between the degree of cannibalism and lake water pH or any other abiotic variable, and the degree of omnivory showed a nonsignificant decline in the lowest pH lakes ($r^2 = 0.15$, $p > 0.10$). The degree of cannibalism did not change because both cannibalistic (piscivorous fish and invertebrate predators) and noncannibalistic predators (planktivorous fish and herbivorous zooplankton) were lost from the webs at roughly the same rate. Omnivory declined at low pH because carnivores (nearly all of which were omnivorous) were lost at a rate that exceeded the losses of strict herbivores.

The ratio of predators to prey and the portion of basal, intermediate, and top species provide a picture of the food web's "shape", i.e. the extent to which the web is dominated by species located at lower versus higher trophic levels. Surprisingly, all four parameters showed no significant relationships with the abiotic variables, including pH (Fig. 3a–3d). A trend that did appear, however, was an increase in the amount of variation in the parameters in the more acid lakes. Briand and Cohen (1984) had previously suggested that the predator/prey ratio and the portions of basal, intermediate, and top species are invariant with S, i.e. that they are fixed constants of all food webs. The present results lend support to that view if only the lakes of pH > 5 are examined. However, the stresses associated with acidification seem to affect the pelagic communities in such a way that the parameters are no longer as predictable. Locke (1992) stated an identical conclusion, i.e. that food web structure becomes unpredictable at pH < 5. In the present study, relationships between the food web parameters and some of the abiotic variables offer a partial explanation. The acid lakes having the highest predator/prey ratio and the greatest portion of top and intermediate species tended to be lower in monomeric Al and higher in DOC and contained fish. In contrast, the acid lakes having low predator/prey ratios and high portions of basal species tended to have higher monomeric Al levels and lower DOC and most were fishless. These relationships were weak, and no significant models could be constructed from the abiotic variables. Nevertheless, the results are consistent with Locke's (1992) view that in lakes of pH < 5, "toxicity becomes an overriding determinant of community structure".

With an increasing level of community stress, the amount of redundancy in a food web is expected to decline (Odum 1985), i.e. there should be fewer species having similar ecological roles. Schindler (1990) noted that in experimentally acidified lakes of the Experimental Lakes Area, Ontario, community-level redundancy declined with pH. This trend has important consequences for community stability because webs with very low redundancy are subject to severe damage when additional species are lost. For example, in Ca-poor Lake 223, a single species of crayfish existed. When it was eliminated during acidification, it "impoverished the food web supporting predatory fishes" (Schindler 1990). In the present study, I determined redundancy as the ratio of biological species to trophic species. The ratio gives the mean number of species sharing each trophic position in the web. Surprisingly, there was no significant decline with pH (Fig. 3e). As with the predator/prey ratio, however, the parameter was more variable in the lower pH lakes. In this case, no relationships with the abiotic variables could be discerned. However, the decline in predictability at low pH is again consistent with the hypothesis of Locke (1992).

In conclusion, acidification was shown to have substantial effects on pelagic food web structure. The number of species declined with pH, as did linkage density, predator generalization, and prey vulnerability, i.e. the food webs of acid lakes were simpler, not only because they contained fewer species but also because the number of biotic interactions per species was lower. Other structural properties of the food webs, including the predator/prey ratio, the portion of basal, intermediate, and top species, and species redundancy, did not decline with pH but were less predictable in the most acid lakes. That finding is consistent with the view that at low pH, toxicity becomes the major determinant of community structure.

Acknowledgements

I am grateful to J. W. Sutherland, New York State Department of Environmental Conservation, for providing the data set used in this study. Comments by A. Locke and S. L. Pimm led to improvements in the manuscript.

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Interactions among Antioxidant Defenses in Liver of Rainbow Trout (*Oncorhynchus mykiss*) Exposed to Cadmium

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Palace, V. P., H. S. Majewski, and J. F. Klaverkamp. 1993. Interactions among antioxidant defenses in liver of rainbow trout (*Oncorhynchus mykiss*) exposed to cadmium. Can. J. Fish. Aquat. Sci. 50: 156–162.

Rainbow trout (*Oncorhynchus mykiss*) (mean weight 180 ± 10 g) fed diets deficient in ascorbic acid, tocopherol, both, or neither were exposed to one of three cadmium concentrations (0, 2, or 4 μg Cd/L). After 181 d of exposure, liver and whole blood were sampled. Cadmium exposure was found to significantly increase the Cd content in liver, especially in fish fed a diet deficient in ascorbic acid. Hepatic stores of ascorbic acid decreased in fish exposed to Cd and in fish fed diets deficient in tocopherol and ascorbic acid. Depletion of tocopherol in liver occurred only in those fish fed a diet deficient in tocopherol and exposed to Cd. Superoxide dismutase activity in liver increased with Cd exposure and with dietary deficiencies of tocopherol and ascorbic acid, while catalase was inhibited by exposure to Cd alone. Glutathione peroxidase activity was unaffected by Cd but was lower in fish fed a diet deficient in both tocopherol and ascorbic acid. Erythrocyte fragility, measured by spontaneous hemolysis of red blood cells in physiological saline, was increased by Cd exposure and the deficiencies of dietary tocopherol and ascorbic acid.

Des truites arc-en-ciel (*Oncorhynchus mykiss*) dont le poids moyen était de 180 ± 10 g ayant été soumises à un régime alimentaire pauvre en acide ascorbique ou en tocophérol, ou pauvre en ces deux éléments, ou encore ne présentant ni une ni l'autre de ces déficiences ont été exposées à l'une des trois concentrations de cadmium suivantes : 0, 2 ou 4 μg Cd/L. Après 181 jours d'exposition, on a prélevé des échantillons de foie et de sang entier. On a observé une augmentation considérable de la teneur en Cd dans le foie par suite d'une exposition au Cd, surtout chez les poissons soumis à un régime alimentaire pauvre en acide ascorbique. Les réserves d'acide ascorbique dans le foie diminuent chez les poissons exposés au Cd et chez les poissons soumis à un régime alimentaire pauvre en tocophérol et en acide ascorbique. Une diminution du tocophérol dans le foie ne survient que chez les poissons soumis à un régime alimentaire pauvre en tocophérol et exposés au Cd. L'activité de la superoxyde-dismutase dans le foie augmente proportionnellement à l'exposition au Cd et aux carences alimentaires en tocophérol et en acide ascorbique, tandis que l'activité de la catalase est inhibée par l'exposition au Cd seulement. L'activité de la glutathion-peroxydase n'est pas modifiée par le Cd, mais elle est moindre chez les poissons soumis à un régime alimentaire pauvre à la fois en tocophérol et en acide ascorbique. La fragilité globulaire, mesurée par l'hémolyse spontanée des globules rouges dans un soluté physiologique, augmente chez les poissons exposés au Cd et soumis à un régime alimentaire pauvre en tocophérol et en acide ascorbique.

Received February 5, 1992
Accepted July 14, 1992
(JB393)

Reçu le 5 février 1992
Accepté le 14 juillet 1992

Cadmium is increasingly recognized as threatening to aquatic systems because of its toxicity to aquatic organisms (Kumada et al. 1980; Hall and Burton 1982; Larsson et al. 1985) and its presence in the environment in toxic concentrations (Hellawell 1988; Nriagu 1988). Previous studies (Majewski and Giles 1981; Giles 1984) exposing rainbow trout (*Oncorhynchus mykiss*) to environmentally relevant concentrations of Cd (3.6 and 6.4 μg/L) have demonstrated disturbances in ionoregulation and oxygen-carrying capacity, possibly indicating alterations in membrane integrity and fluidity.

Membrane fluidity is essential for a variety of cellular functions and is maintained through incorporation of polyunsaturated fatty acids (PUFA) in membrane lipids. Due to the presence of high numbers of methylene-interrupted double bonds, PUFA are susceptible to breakdown through a radical-generated process known as lipid peroxidation (Horton and Fairhurst 1987). Cadmium has been identified as a potential lipid peroxidation inducer for PUFA (Stacey et al. 1980) which may be important for fish because they are particularly rich in PUFA lipids (Hearn et al. 1987; Gibson 1989).

Enzymatic defenses against lipid peroxidation include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). These three enzymes inhibit lipid peroxidation by detoxifying reactive oxygen species generated by the process throughout the cell (Mather-Mihaich and DiGiulio 1986). Chronic exposure to environmental pollutants such as cyanide and Cd has been shown to affect the activities of these enzymes in mammals, corresponding to an increase of lipid peroxidation (Stacey et al. 1980; Ardelt et al. 1989).

Nonenzymatic antioxidants including tocopherol and ascorbic acid are also important for defense against lipid peroxidative breakdown of membrane lipids. Lipid radicals that arise from exposure to environmental pollutants and that propagate lipid peroxidation are detoxified by tocopherols (Mezes and Vadasz

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1984). As one of its physiological functions, ascorbic acid may regenerate tocopherol from the tocopheroxy radical that is produced during the initial detoxification of peroxidizing lipids (Geetha et al. 1989). Ascorbic acid may possess other antioxidant activities. Koul et al. (1988) have suggested that ascorbic acid may directly scavenge free radicals or maintain other aqueous-soluble antioxidants in the reduced state. Recent work has shown that only after ascorbic acid is consumed may peroxidation occur in the lipid phase (Krinsky 1988). Neff (1985) indicated that pollutant stresses may result in depletion of one or both of these nonenzymatic defense parameters.

Metallothionein, a low molecular weight protein characterized by high cysteine and metal content (Noel-Lambot et al. 1978), may be indirectly effective in reducing lipid peroxidative membrane damage which subsequently results in acclimation to metal toxicity (Klaverkamp et al. 1984). Metallothionein may sequester heavy metals away from sites of potential toxic action. Included in these sites are membrane and cytosolic enzymes (Jenkins et al. 1982) such as SOD, CAT, and GPx. In addition, metallothionein may prevent metals from contacting membrane lipids where they can induce lipid peroxidation (Andersen and Andersen 1988). More recently, it has been suggested that metallothionein may play a more direct role in preventing peroxidative damage by scavenging free radicals (Shi 1990).

Increased lipid peroxidation may be manifested by the appearance of a variety of cellular alterations. One that is easily measured is the disturbance of membrane fluidity (Leighton et al. 1985) which can be quantified by measuring the relative ease of lysis of erythrocytes (Draper and Csallany 1969).

The objectives of this study included an examination of effects of Cd exposure (2 and 4 μg/L) and dietary deficiencies (tocopherol and ascorbic acid) on the status of hepatic enzymatic and nonenzymatic antioxidants. Hemolysis was used to measure the extent of oxidative erythrocyte membrane damage produced by Cd and dietary deficiencies.

Materials and Methods

Rainbow trout (mean weight 182 ± 4 g) were acclimated in 140-L fiberglass tanks at 12 ± 1°C in dechlorinated Winnipeg city tap water (Ca = 20–25 mg/L, hardness as $CaCO_3$ = 80 mg/L, conductivity = 165 μmho/cm, pH = 7.8) for 30 d prior to Cd exposure. Water exchange in each tank was at a rate of 1 L/min providing a 95% replacement time of approximately 7 h. Each tank contained a group of 20 fish. Fish were fed 1% of body weight every second day for the first 14 d and starved for the remaining 16 d of the acclimation period. When feeding occurred, tanks were flushed to remove uneaten food and feces 1 h after each feeding period. A 12 h light : 12 h dark photoperiod was maintained throughout the acclimation and exposure periods. Following acclimation, four randomly selected groups were exposed to 2 μg Cd/L (from $CdCl_2$, actual concentration 1.82 ± 0.03 μg/L). Similarly, four groups were exposed to 4 μg Cd/L (3.96 ± 0.35 μg/L). Four unexposed groups served as controls. Therefore the experiment included a total of 12 tanks containing 20 fish each. Cadmium solution was constantly added to the inflowing water by a peristaltic pump. No mortalities were recorded in any of the tanks throughout the experiment.

The groups of 20 fish were randomly assigned and fed one of four diets: tocopherol deficient, ascorbic acid deficient, tocopherol and ascorbic acid deficient, or a reference diet deficient in neither ascorbic acid nor tocopherol. A modification of the diet of Dabrowski et al. (1990) was used as described in Table 1. For diet formulation, ingredients were mixed dry except for those noted with an asterisk in Table 1 which were suspended in 200 mL of double-distilled water. Ingredients were mixed in a Hobart mixer with an additional 750 mL of water to form a paste that was extruded through a pelletizer of 2.0-mm diameter. The strands were cut into approximately 1-cm-long pellets, frozen, and stored at −120 ± 10°C (Hilton et al. 1977). The appropriate ration was removed from storage daily, thawed, and fed immediately. Pellets were presented by slowly sprinkling on top of the water surface so that they were completely consumed in less than 5 s of contact with the water.

TABLE 1. Formulation of basal diet fed to rainbow trout.

Ingredient	g/kg
Dextrin	157.31
Gelatin	60.08
Casein	581.29
Cellulose	27.94
Arginine	10.01
Methionine	5.01
Palmitic acid	90.12
Linolenic acid	0.14
Vitamin premix[a]	28.04
Mineral premix[b]	40.06

[a]Vitamin premix (*indicates ingredients that were suspended in 200 mL of water before mixing):
*thiamine hydrochloride = 0.115 g, *riboflavin = 0.460 g, *pyridoxine hydrochloride = 0.115 g, niacin = 1.720 g, calcium pantothenoate = 1.150 g, myoinositol = 4.590 g, *biotin = 0.011 g, *folic acid = 0.035 g, choline chloride = 19.714 g, *menadione = 0.090 g, and *cyanocobalamin = 0.002 g. Ascorbic acid where applicable was added at 1.0 g/kg and tocopherol acetate at 0.400 g/kg.

[b]Mineral premix (*indicates ingredients that were resuspended in 200 mL of water before mixing):
$Ca(H_2PO_4)_2$ = 27.5 g, $CaCO_3$ = 2.1 g, $MgSO_4 \cdot 7H_2O$ = 3.6 g, $FeSO_4 \cdot 7H_2O$ = 1.2 g, KCl = 2.0 g, NaCl = 3.2 g, *$Al_2(SO_4)_3$ = 0.008 g, *$ZnSO_4 \cdot 7H_2O$ = 0.16 g, *$CuSO_4 \cdot 5H_2O$ = 0.04 g, *$MnSO_4 \cdot 4H_2O$ = 0.14 g, *KI = 0.009 g, *Na_2SeO_3 = 0.003 g, and *$CoSO_4 \cdot 7H_2O$ = 0.04 g.

Ascorbic acid and tocopherol acetate, as well as all other diet constituents, were purchased from Sigma Chemical Company, St. Louis, MO. Each group was fed 36.0 g/day of the appropriate diet, approximately 1% of body weight, for a period of 181 d. Although growth was not measured, there were no differences in condition factor for any of the groups of fish (p = 0.05). At the end of this period, fish were anesthetized for 5 min in pH-buffered tricaine methanesulphonate (MS222) (Wedemeyer 1970; Palace et al. 1990). Blood was obtained through caudal vein puncture using a 1-in. 22-gauge needle attached to a 1-mL syringe. All livers were dissected, placed in sterile plastic bags, and immediately frozen on dry ice and stored at −120 ± 10°C to avoid biochemical breakdown (Palace et al. 1990).

All biochemical parameters were analyzed in random fashion from the tissues collected. Superoxide dismutase, CAT, and GPx were assayed at 12 ± 1°C using the methods of Sun and Zigman (1978), Rabie et al. (1972), and Tappel (1978), respectively. Total ascorbic acid was determined using the method of Jagota and Dani (1982). Total tocopherol was analyzed using the extraction method of King (1985) followed by the colorimetric procedure of Tsen (1961). Metallothionein was determined by the mercury displacement method described by Klaverkamp et al. (1991).

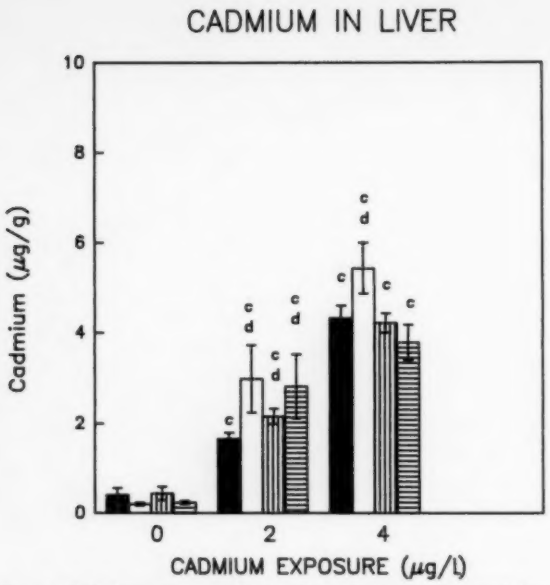

FIG. 1. Cadmium concentrations in liver. Data are expressed (mean with standard error) (as μg/g wet weight ($n = 7, p = 0.05$). c = Cd effects, i.e. significant differences between groups receiving the same diet at different cadmium exposures; d = diet effects, i.e. significant differences from the reference diet group at the same cadmium exposure. ■, reference diet; □, ascorbic acid deficient; ▥, tocopherol deficient; ▤, ascorbic acid and tocopherol deficient.

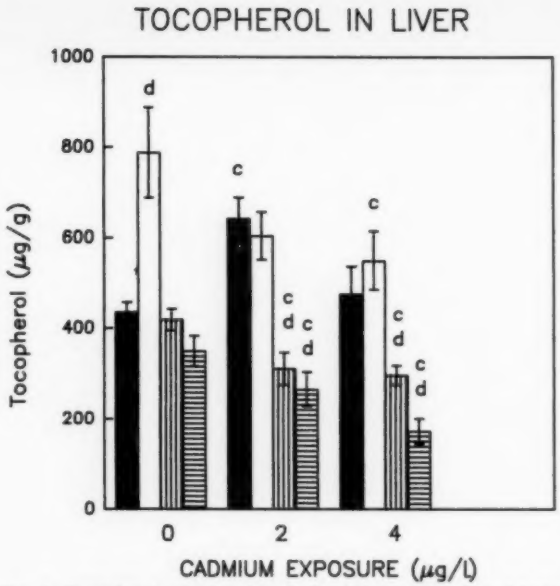

FIG. 2. Tocopherol concentrations in liver. Data are expressed (mean with standard error) as μg/g wet weight ($n = 7, p = 0.05$). Cadmium (c) and diet (d) effects as in Fig. 1; graphic notation for the various diets as in Fig. 1.

All data were analyzed for statistical significance using ANOVA and differences between specific means verified using Student's *t*-test. Statistical significance was set at $p = 0.05$.

Results and Discussion

In our laboratory, Cd has been shown to have an incipient lethal level (ILL) in rainbow trout of 16 μg Cd/L (Majewski and Giles 1981). Our experimental exposures of 2 and 4 μg Cd/L correspond to 13 and 25%, respectively, of this ILL. After 181 d, Cd accumulated significantly in liver and correlated positively with Cd exposure for all diets (Fig. 1). This accumulation occurred even though the exposures took place in relatively hard water where Ca is known to inhibit Cd uptake (Wicklund and Runn 1988). Fish fed diets deficient in ascorbic acid accumulated more hepatic Cd than fish fed the reference diet (Fig. 1). Rambeck et al. (1988) also demonstrated elevated Cd uptake in ascorbic acid deficient chickens. They suggested that adequate stores of ascorbic acid inhibit Cd accumulation by increasing absorption of Fe which competes with Cd for cellular binding and transport sites. There were no consistent significant differences in Cd accumulation between fish fed diets deficient in tocopherol and fish receiving dietary tocopherol. Additionally, the diet deficient in tocopherol and ascorbic acid failed to show the same accumulation of Cd as the diet deficient in ascorbic acid at the highest Cd exposure.

Cadmium had no consistent effects on liver tocopherol for fish fed the reference diet (Fig. 2), although liver tocopherol concentrations were greater for fish fed the reference diet and exposed to 2 μg Cd/L (Fig. 2). Tocopherol concentrations did decline in the presence of Cd and dietary deficiency of tocopherol compared with the fish fed the reference diet (Fig. 2). Tocopherol concentrations were expected to decline, as Cd has been reported to induce lipid peroxidation by producing lipid hydroperoxides (Stacey et al. 1980) which consume tocopherol (Stocker and Peterhans 1989). Consumption of tocopherol by hydroperoxides in Cd-exposed fish, coupled with dietary deficiency of the vitamin, likely contributed to reduced tissue stores of tocopherol. None of the other diet treatments consistently affected tocopherol concentrations.

Ascorbic acid concentrations were significantly depleted in fish exposed to Cd and fed the reference diet (Fig. 3). Consumption of diets deficient in ascorbic acid led to further depletion of hepatic concentrations of ascorbic acid (Fig. 3). In the absence of Cd, ascorbic acid stores in liver were significantly ($p = 0.05$) depleted only in fish fed diets deficient in both ascorbic acid and tocopherol. Without the added demand for ascorbic acid with Cd exposure, hepatic ascorbic acid was not significantly lower in fish fed a diet deficient in ascorbic acid.

Both Cd exposure and dietary deficiency of ascorbic acid would be expected to reduce tissue stores of ascorbic acid. Because of a lack of L-gulonolactone oxidase enzyme activity, most teleost fish are unable to synthesize ascorbic acid (Chatterjee 1973), and therefore, diets deficient in ascorbic acid would result in a decrease in tissue concentrations of the vitamin. Chronic exposure to Cd induces hyperglycemia in striped mullet (*Mugil cephalus*), and because high glucose concentrations can reduce uptake of ascorbic acid, tissue stores of ascorbic acid may be further depleted in fish exposed to Cd (Thomas 1987). Thomas (1987) postulated that decreases in ascorbic acid are part of a nonspecific stress response. The response involves hyperglycemia and the release of adrenocorticotropic hormone (ACTH) from the pituitary, thereby stimulating cortisol production from the interrenal gland (Wedemeyer 1969). An experiment by Wedemeyer (1969), in which stressed coho salmon (*Oncorhynchus kisutch*) showed reduced kidney ascorbic acid but no concurrent rise in plasma ascorbic acid, is seen as evidence for participation by ascorbic acid in cortisol production.

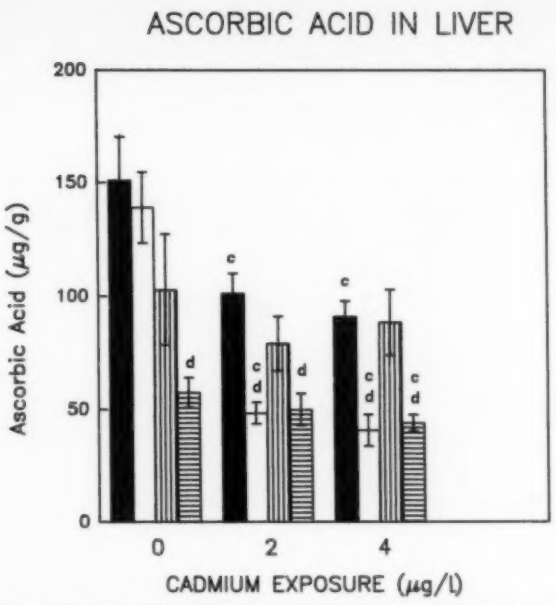

FIG. 3. Ascorbic acid concentrations in liver. Data are expressed (mean with standard error) as μg/g wet weight ($n = 7$, $p = 0.05$). Cadmium (c) and diet (d) effects as in Fig. 1; graphic notation for the various diets as in Fig. 1.

TABLE 2. Metallothionein content in livers of fish exposed to various concentrations of Cd and fed either experimental or reference diets. Data are expressed as mean ± SE ($n = 7$). c = Cd effects, i.e. significant differences between groups receiving the same diet at different [Cd] ($p = 0.05$); d = diet effects, i.e. significant differences from the reference group at the same [Cd] ($p = 0.05$).

Diet deficiency	Cd exposure (μg/L)	Metallothionein in liver (μg/g)
Reference	0	0.76 ± 0.24
	2	0.57 ± 0.16
	4	0.54 ± 0.05
Ascorbic acid	0	0.60 ± 0.24
	2	0.57 ± 0.20
	4	0.35 ± 0.04 (d)
Tocopherol	0	0.60 ± 0.20
	2	0.35 ± 0.12
	4	0.49 ± 0.09
Tocopherol + ascorbic acid	0	0.27 ± 0.05 (d)
	2	0.55 ± 0.11 (d)
	4	0.32 ± 0.05 (d)

Whether elevated cortisol is responsible for or simply accompanies the resultant hyperglycemia is unclear; however, the condition can significantly deplete ascorbic acid stores (Neff 1985).

Depletion of ascorbic acid with exposure to Cd may also be due to increased usage of ascorbic acid as an antioxidant. Ascorbic acid may trap peroxides in the aqueous layer before they can diffuse into the lipid layer and induce lipid peroxidation (Frei et al. 1989). Additionally, ascorbic acid can act as a link between the aqueous antioxidant fraction of the cell and the lipid fraction by regenerating tocopherol in a complex electron transport system (Stocker and Peterhans 1989). A peculiarity of this proposed electron transport system is that ascorbic acid may act as an oxidant at physiological concentrations when tocopherol is depleted (Staats and Colby 1989). Therefore, a deficiency of tocopherol is damaging not only because of the loss of antioxidant activity, but also because of additional oxidant activity from ascorbic acid. However, other investigators report greater dependence on ascorbic acid as an antioxidant during tocopherol deficiency. For example, tocopherol-deficient rats have been shown to increasingly utilize ascorbic acid, presumably to compensate for the antioxidant activity lost from tocopherol (Pentyuk et al. 1989). The reduction in liver ascorbic acid in fish fed the diet deficient in both ascorbic acid and tocopherol and not exposed to Cd (Fig. 3) supports this theory.

It is well established that metallothionein synthesis increases with exposure to Cd in gulls, humans, and fish (Leighton et al. 1985; Klaverkamp and Duncan 1987; Bem et al. 1989). However, no significant increases in metallothionein concentrations were found in liver of fish exposed to Cd (Table 2). Metallothionein concentrations were significantly ($p = 0.05$) lower in fish fed diets deficient in ascorbic acid and exposed to 4 μg Cd/L and fish fed diets deficient in ascorbic acid and tocopherol even without Cd exposure (Table 2). There were no significant differences in liver metallothionein of fish fed diets deficient in tocopherol. The role of and dependency on ascorbic acid in the synthesis and metabolism of metallothionein in fish need to be investigated further. Analyses of in situ metals in metallothionein-containing fractions of cellular cytosols from Cd-exposed fish are also required to determine whether Cd was simply displacing metals, such as Zn, with weaker binding affinities (Klaverkamp and Duncan 1987). Investigations in mammals have demonstrated that dietary supplementation of ascorbic acid induces metallothionein synthesis (Onasaka et al. 1987). This may indicate a role for ascorbic acid in metallothionein synthesis and may explain why fish fed diets deficient in ascorbic acid actually showed a decline of metallothionein concentration even when Cd was present.

Glutathione peroxidase enzyme activity was not significantly different in fish fed the reference diet and exposed to Cd (Fig. 4). Glutathione peroxidase activity was significantly lower for all fish fed diets deficient in tocopherol and ascorbic acid (Fig. 4). Previous studies in rats and humans have also demonstrated lower glutathione peroxidase activity during tocopherol deficiencies (Jain et al. 1988; Hill et al. 1989) and during ascorbic acid deficiency in guinea pigs (Koul et al. 1988). More study of fish is required to determine if ascorbic acid and tocopherol are directly involved in GPx sythesis or activation or whether these compounds affect the activity of the enzyme indirectly through cellular control of antioxidant equivalents.

Among fish fed the reference diet, SOD activity was greatest in fish exposed to 4 μg Cd/L (Fig. 5). Enhancement of SOD activity may indicate that increased lipid peroxidation was occurring and that there was simultaneous demand for increased antioxidant enzymatic activities within the cell (Mather-Mihaich and DiGiulio 1986). Similar increases in SOD activity have been documented among feral spot (*Leiostomus xanthurus*) collected from an estuary polluted by metals and organochlorines (Roberts and Sved 1987).

At both Cd concentrations, hepatic SOD activity was higher in fish fed diets deficient in both ascorbic acid and tocopherol than in fish fed the reference diet (Fig. 5). Koul et al. (1988),

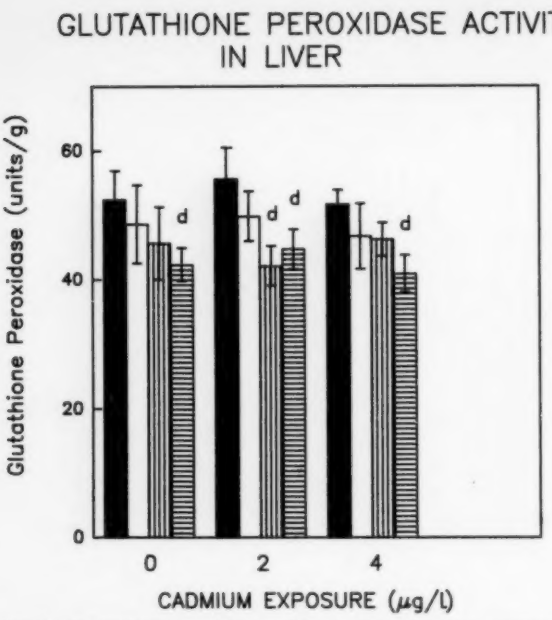

FIG. 4. Glutathione peroxidase activity in liver. Data are expressed (mean with standard error) as units/g wet weight. One unit of GPx catalyzes the oxidation of 1.0 μmol of reduced glutathione by H_2O_2 to oxidized glutathione per minute at pH = 7.0, 25°C (n = 7, p = 0.05). Cadmium (c) and diet (d) effects as in Fig. 1; graphic notation for the various diets as in Fig. 1.

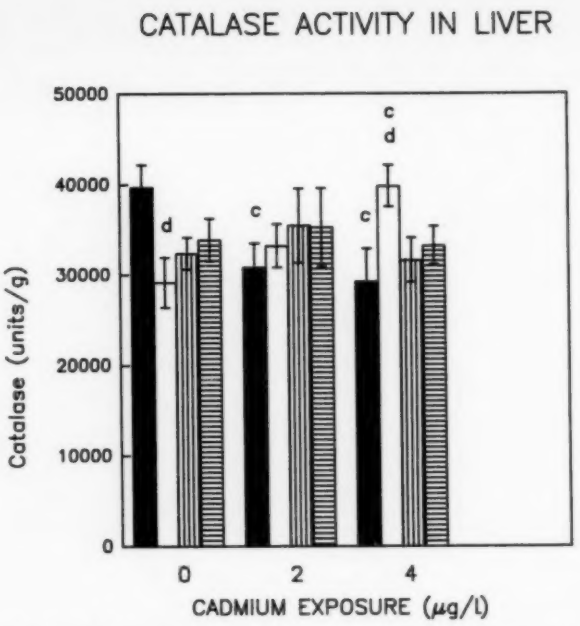

FIG. 6. Catalase activity in liver. Data are expressed (mean with standard error) as units/g wet weight. One unit of catalase decomposes 1.0 μmol of H_2O_2 at pH = 7.0, 25°C (n = 7, p = 0.05). Cadmium (c) and diet (d) effects as in Fig. 1; graphic notation for the various diets as in Fig. 1.

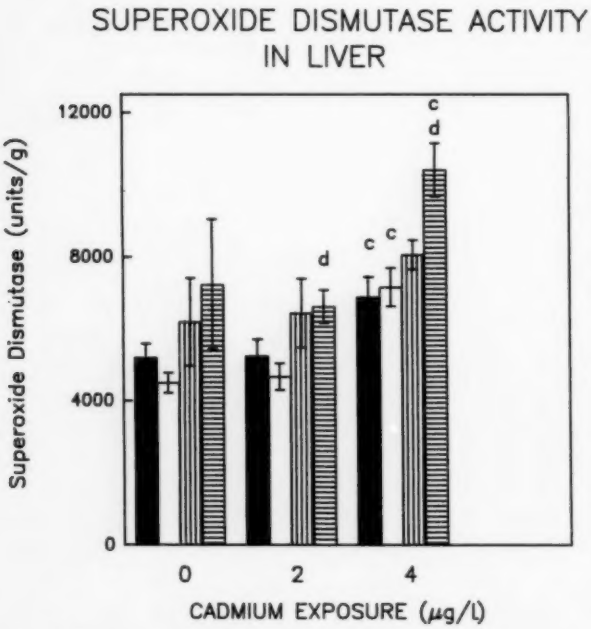

FIG. 5. Superoxide dismutase activity in liver. Data are expressed (mean with standard error) as units/g wet weight. One unit of SOD inhibits oxidation of epinephrine by 50% at pH = 7.0, 25°C (n = 7, p = 0.05). Cadmium (c) and diet (d) effects as in Fig. 1; graphic notation for the various diets as in Fig. 1.

however, reported decreased SOD activity in the guinea pig with ascorbic acid deficiency. Superoxide dismutase activity induction has been reported in rats fed diets deficient in tocopherol (Hill et al. 1989). Because ascorbic acid and tocopherol serve as antioxidants against lipid peroxidation, their exclusion along with Cd exposure probably increases demand for enzymatic antioxidants, such as SOD.

Catalase activity was less in fish fed the reference diet and exposed to Cd compared with those fish fed the reference diet and not exposed to Cd (Fig. 6). Catalase inhibition with exposure to heavy metals is consistent with previous work (Singh and Sivalingam 1982; Oh et al. 1989). Possible mechanisms by which Cd produces lower CAT activity include direct metal-mediated structural alteration of the enzyme (Arillo et al. 1984) and depression of CAT synthesis (Pruell and Engelhardt 1980).

Although CAT activity was varied slightly in fish fed the diet deficient in ascorbic acid, no consistent alterations of CAT activity were evident in any of the diet treatments compared with the fish fed the reference diet (Fig. 6). Previous mammalian work has shown that CAT activity declines in ascorbic acid deficient guinea pigs (Koul et al. 1988). Recent work by Geetha et al. (1989) also showed higher liver and heart CAT activity in rats receiving oral supplements of tocopherol. They hypothesized that the higher tocopherol concentrations offer an alternative antioxidant source, thereby sparing the activity of antioxidant enzymes such as CAT.

Measuring the relative ease of hemolysis of erythrocytes is useful as an indicator of the extent of lipid peroxidative damage on cellular membranes and as an indicator of overall cellular antioxidant performance (Berry et al. 1988). The use of such measures as indicators of pollutant exposure in fish has been previously validated (Srivastava and Narain 1982). Samples from fish exposed to 2 μg Cd/L were mishandled and are there-

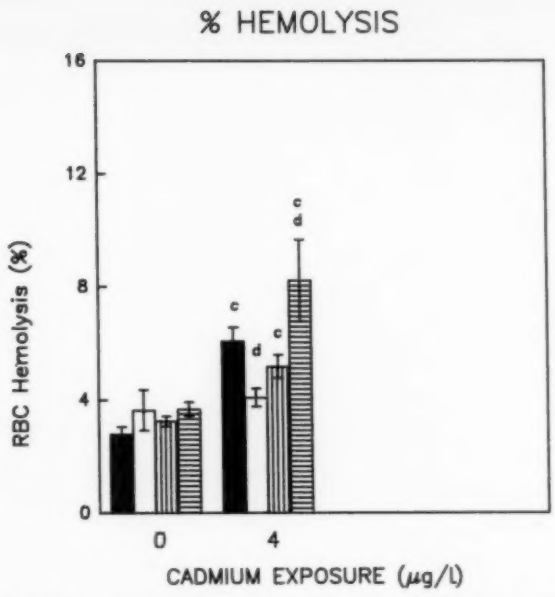

FIG. 7. Spontaneous hemolysis of erythrocytes in physiological saline. Data are expressed (mean with standard error) as a percentage of the total red blood cell population, based on optical density, in 0.3 mL of whole blood ($n = 7$, $p = 0.05$). Cadmium (c) and diet (d) effects as in Fig. 1; graphic notation for the various diets as in Fig. 1.

fore not presented here; however, red blood cell hemolysis was significantly increased with exposure to 4 μg Cd/L for three of the four diets (Fig. 7). This hemolysis may indicate increased peroxidation of erythrocyte membranes because of Cd-induced depletion of enzymatic and nonenzymatic antioxidant capabilities. Ascorbic acid and tocopherol deficiency coupled with Cd exposure led to higher spontaneous hemolysis, indicating lipid peroxidative weakening of the plasma membranes (Berry et al. 1988). Previous work has shown similar results for tocopherol-deficient rats (Willis 1983).

In summary, all three antioxidant enzymes measured in the liver of rainbow trout have shown some utility for assessing oxidant stress from Cd exposure and dietary deficiencies of ascorbic acid and tocopherol. Superoxide dismutase appears to be the most sensitive of the three, showing higher activities with both Cd exposure and dietary deficiencies of the vitamins. Given the decline of ascorbic acid in rainbow trout liver, seen here with Cd exposure, and the contribution of ascorbic acid in ameliorating the toxicity of Cd, it seems especially important to consider ascorbic acid tissue stores when assessing the potential of Cd as an initiator of lipid peroxidation. Tocopherol, although more resistant to decline than ascorbic acid, must also be considered where both dietary deficiency and Cd toxicity are a concern. Hemolysis of red blood cells is an integrative indicator of overall antioxidant performance and oxidative stress due to Cd exposure and dietary deficiencies of ascorbic acid and tocopherol. Because metallothionein was measured only at the end of the experiment, we have no indication of the initial induction phase of the protein with Cd exposure. However, after 181 d of exposure to Cd, metallothionein actually declined in some fish fed diets deficient in ascorbic acid. The possibility remains that metallothionein may act as a scavenger of free radicals (Shi 1990) when other antioxidants are absent. These observations are especially relevant because the concentrations of Cd achieved here in rainbow trout livers are similar to those found in livers of natural populations of fish exposed to atmospheric deposition of Cd and other metals (Harrison and Klaverkamp 1990).

Acknowledgements

The authors wish to thank S. H. Harrison and J. Tabachek for their review of the preliminary manuscript and E. Schindler for her technical assistance. This work was supported by a Department of Fisheries and Oceans – NSERC science subvention grant administered by the University of Manitoba.

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Relationships between Prey Concentration and Growth, Condition, and Mortality of Pacific herring, *Clupea pallasi*, Larvae in an Alaskan Subarctic Embayment

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McGurk, M. D., A. J. Paul, K. O. Coyle, D. A. Ziemann, and L. J. Haldorson. 1993. Relationships between prey concentration and growth, condition, and mortality of Pacific herring, *Clupea pallasi*, larvae in an Alaskan subarctic embayment. Can. J. Fish. Aquat. Sci. 50: 163–180.

We tested the hypothesis that growth, condition, and mortality of four "trace" cohorts of Pacific herring, *Clupea pallasi*, larvae in Auke Bay, Alaska, in 1988 were limited by concentration of prey. Hatching of herring larvae coincided with the spring maximum of copepod nauplii production, so prey concentration was high enough to support successful feeding and growth. However, growth and condition differed significantly among cohorts. Low growth and condition were not caused by density-dependent competition for food among herring larvae because larvae were too dilute to affect prey concentration and were unlikely to be caused by competition within the entire plankton community because there is no evidence that nonherring plankters interfered with feeding of herring larvae. Most larvae with low condition were from two cohorts that hatched from the upper zone, which suggests that desiccation and extremes of temperature may have disrupted embryonic development and produced larvae that were not competent to feed successfully. There were no among-cohort differences in instantaneous mortality. We conclude that growth, condition, and mortality of herring larvae in Auke Bay in 1988 were not limited by food, but by other factors such as predation and environmental conditions during egg incubation.

Nous avons étudié l'hypothèse selon laquelle en 1988, la croissance, la condition physique et la mortalité chez quatre petites cohortes de larves de hareng du Pacifique, *Clupea pallasi*, établies dans la baie Auke (Alaska) auraient été limitées en raison de la concentration des proies. L'éclosion printanière des larves de hareng ayant coïncidé avec une production maximale de nauplii de copépodes, la concentration des proies était suffisamment élevée pour assurer le succès de la croissance et de l'alimentation chez les larves. Toutefois, on observe des variations importantes de la croissance et de la condition physique dans les différentes cohortes. Chez les larves de hareng, la faible croissance et la condition physique médiocre ne sont pas imputables à une compétition pour la nourriture due à la densité, les larves étant trop dispersées pour influer sur la concentration des proies; en outre, ces conditions ne résultaient probablement pas d'une compétition dans l'ensemble de la communauté planctonique, car il n'y a pas de preuves que les organismes planctoniques autres que les larves de hareng auraient nui à l'alimentation de ces dernières. La plupart des larves qui présentaient une condition physique médiocre appartenaient à deux cohortes provenant d'oeufs éclos dans la partie supérieure de l'estran, ce qui laisse penser que le dessèchement et les températures extrêmes peuvent avoir perturbé le développement embryonnaire, provoquant ainsi la naissance de larves inaptes à s'alimenter avec succès. On n'observe pas de différence entre les cohortes sur le plan de la mortalité instantanée. Nos conclusions sont que la croissance, la condition physique et la mortalité chez les larves de hareng de la baie Auke en 1968 n'étaient pas limitées du fait de la nourriture, mais à cause d'autres facteurs, notamment la prédation et les condition du milieu au cours de la période d'incubation des oeufs.

Received May 3, 1991
Accepted July 7, 1992
(JB022)

Reçue le 3 mai 1991
Accepté le 7 juillet 1992

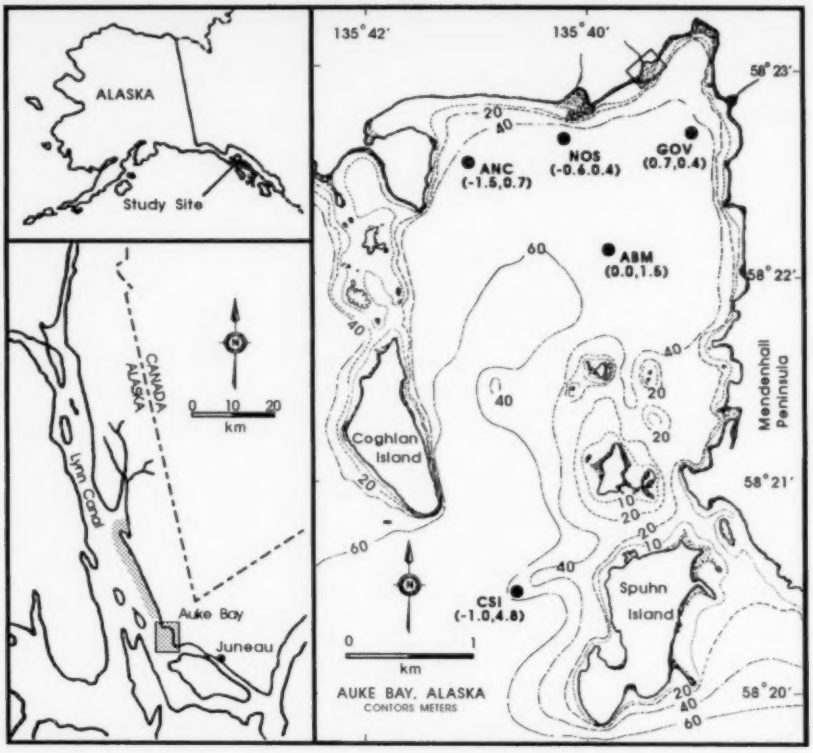

FIG. 1. Maps of Auke Bay showing it in relation to Alaska and Lynn Canal. The stippled area along the eastern shore of Lynn Canal was the major spawning area for the Auke Bay – Lynn Canal herring stock in 1988. Closed circles in Auke Bay are the plankton sampling stations and their x–y coordinates (in units of kilometres). Depth contours are in metres. The boxed area on the north shore of Auke Bay was the spawning beach for herring cohorts 2 and 3. Only Waydelich Creek is shown entering the spawning beach.

Little is known of the population dynamics of Pacific herring, *Clupea pallasi*, larvae in Alaska, which is significant because year-class strength of fish populations is probably established during early life history. This gap in knowledge stimulated recent larval herring surveys in southeast Alaska (Haldorson and Collie 1991), southcentral Alaska (McGurk et al. 1990; Smith et al. 1991), and the southeast Bering Sea (McGurk 1991). This paper is the first comprehensive analysis of growth, condition, and mortality of herring larvae in Alaska.

The specific hypothesis of our study was that timing of the spring bloom of microzooplankton prey, and variation in prey concentration during the spring bloom, controls growth and survival of larval herring. This hypothesis was first proposed for Atlantic herring, *Clupea harengus*, larvae by Hjort (1914) and was subsequently elaborated by Cushing (1975, 1990) as the match–mismatch hypothesis — the idea that growth and survival of herring larvae vary with the degree of temporal match between hatching of larvae and the spring bloom of their prey. Of course, prey concentration is only one of several factors, including extremes of temperature and salinity, predation, and disease, that control larval growth and survival. These factors occur simultaneously in what Fortier and Gagné (1990) called a "hierarchy of constraints". However, prey concentration is believed by many researchers to be at the top of the list of constraints because it is vital to growth and because it is a dynamic variable that links fish larvae with the physical and biological variability of their environment. Recent research has identified density-dependent competition for food between herring larvae (Kiørboe et al. 1988; Fortier and Gagné 1990) and competition for food among fish larvae and the entire community of carnivorous plankton (Fortier and Harris 1989) as processes that may modify the match–mismatch hypothesis.

Previous research in British Columbia waters has not been conclusive on the hierarchy of constraints to survival of Pacific herring larvae. Extremes of temperature and salinity during incubation of eggs affect the production of viable larvae in the laboratory (Alderdice and Velsen 1971; Alderdice and Hourston 1985) and in nature (Purcell et al. 1990), but little is known of the role of physical factors in population regulation. Stevenson (1962) concluded from the distribution of herring larvae in Barkley Sound, Vancouver Island, that the primary factor controlling year-class size was dispersal of herring larvae from food-rich nearshore habitat to food-poor offshore habitat. Recently, Robinson and Ware (1988) and McGurk (1989) confirmed starvation in natural populations of Pacific herring larvae using RNA–DNA ratios and morphometry, respectively. Several authors, noting the high densities of invertebrate predators found near herring spawning areas, and the fact that sea-caught herring larvae often appear to be successfully feeding and growing, argued that predation must play an important part (Arai and Hay 1982; Alderdice and Hourston 1985; Purcell and Grover 1990).

We tested the match–mismatch hypothesis by comparing timing of hatch of four cohorts of herring larvae with timing of the spring bloom of herbivorous zooplankton. Growth of herring larvae was measured from analyses of length frequencies and from the number and widths of increments in the otoliths of herring larvae. Condition was measured with a morphometric condition factor, and mortality was measured from the decline in number of herring larvae, after adjusting for losses due to dispersal of larvae from the study area.

Study Site

Auke Bay (58°22′N, 134°40′W) is a small (11.5-km^2 area) semienclosed bay located approximately 20 km north of Juneau, Alaska (Fig. 1). It has steep sides which fall to an average depth of about 40 m. A deep channel along the east shore of Coghlan Island is the primary entrance for tidal flows; all other channels are less than 20 m deep. Auke Bay receives freshwater directly from four small streams and indirectly from the Mendenhall River 4 km east of the Bay.

Auke Bay is stratified from May to August with a pronounced pycnocline at about 20 m (Coyle and Shirley 1990; Ziemann et al. 1990a). Frequent exchange of water between Auke Bay and the surrounding fjord system leads to the replacement of at least 300% of Auke Bay's volume from May to August (Nebert 1990). Regional weather events that affect upwelling and strong (>4 m·s^{-1}) local winds may be the primary variables responsible for exchange.

There is no consensus on the general pattern of surface currents in Auke Bay. Research in the 1970s and 1980s that used current meters placed close to shore for periods of 1–2 wk suggested that a counterclockwise gyre formed each summer when surface water flowed in Auke Bay between Spuhn Island and Mendenhall Peninsula and flowed out between Coghlan Island and the north shore and between Coghlan and Spuhn islands (Coyle and Shirley 1990). However, recent work by Nebert (1990), who placed four current meters along the west shore of the Bay from April to July in 1988 and 1989, showed the opposite pattern — a general clockwise circulation with water entering the Bay from the deep channel east of Coghlan Island and curving eastward as it approached the northeastern corner of the Bay. Currents were episodic and sometimes reached 0.05 m·s^{-1} for several days at a time.

An intense diatom bloom usually starts in early April and ends one month later (Ziemann et al. 1990b). One or two secondary blooms, caused by nutrient resupply due to wind-driven mixing, follow in May or June. From mid-May to mid-June, zooplankton population numbers reach maxima of 10 000 to 18 000·m^{-3} (Coyle and Paul 1990b). Zooplankton numbers are dominated by herbivorous copepods, and the *Pseudocalanus* species complex is the dominant type of copepod. Invertebrate predators of copepods include chaetognaths, pelagic amphipods, jellyfish, ostracods of the genus *Conchoecia*, and athecate pteropods; the first three groups are also the main invertebrate predators of herring larvae.

Fish larvae of Auke Bay can be divided into three groups depending on whether they hatch before, during, or after the herbivorous copepod maximum. The four most common species that hatch during the copepod maximum are walleye pollock, *Theragra chalcogramma*, flathead sole, *Hippoglossoides elassodon*, eulachon, *Thaleichthys pacificus*, and herring (Haldorson et al. 1987, 1988, 1989, 1990). Walleye pollock and eulachon are the most abundant, and herring is always the least abundant. The primary diet item of first-feeding larvae of all four species is copepod nauplii.

Herring larvae are a minor constituent of the larval fish community of Auke Bay because the Bay is currently the southernmost spawning site for the Lynn Canal herring stock (Carlson 1980). From 1953 to 1960, most of this stock spawned in Auke Bay, but since 1972, spawning has shifted 10–15 km north to the shore of Lynn Canal. Auke Bay has become a marginal site, receiving trace amounts of spawn or no spawn at all (D. Ingledue, Alaska Department of Fish and Game, Commercial Fisheries Division, P.O. Box 240020, Douglas, AK 99824-0020, pers. comm.). SCUBA surveys of Lynn Canal have shown that herring eggs are usually distributed in the intertidal and subtidal zones between +3.7 and −11.6 m from mean lower low water (Blankenbeckler and Larson 1982, 1985, 1987; Blankenbeckler 1987).

Materials and Methods

Temperature and salinity profiles of Auke Bay from March to late June were measured at Auke Bay Monitor (ABM) station with a conductivity – temperature – depth meter (Applied Microsystems, Inc.) that was lowered to a depth of 40 m, equilibrated for 1 min, and then retrieved at a speed of approximately 5 m·min^{-1} (Fig. 1). ABM was chosen to represent Auke Bay because it lies in the center of the Bay and because Auke Bay is small enough that only one station was needed to adequately characterize the hydrography of the Bay. Temperature profiles from late June to mid-August were measured at ABM with a single temperature–depth probe. Salinity profiles for the same time period are not reported because the salinity probe used after day of year (DOY) 174 performed erratically.

Total daily solar irradiance was measured with a quantum solar irradiance meter located on the roof of the Center for Fisheries and Ocean Sciences, University of Alaska, which is on the shore of Auke Bay. Air temperatures were taken from records of the Juneau airport (58°22′N, 134°35′W) and tide heights were taken from tide tables (U.S. Department of Commerce 1988a, 1988b).

To locate herring spawning beaches, aerial surveys of the Auke Bay – Lynn Canal area were conducted every 1–2 d from April 22 to May 15, 1988, and Auke Bay beaches were searched by foot once each week from April 30 to June 19, 1988. Length and width of herring spawn were measured with a rope marked in 1-m intervals. On two dates, samples of spawn were taken at five sites on the spawning beach. All vegetation and attached spawn were collected from within a 0.1-m^2 sample frame and immediately preserved in Gilson's fluid. The number of eggs in each sample was estimated volumetrically: the volume of each sample was measured as the amount of water it displaced, the number of eggs in each of two 5-mL subsamples was counted under a dissecting microscope, and the average number of eggs per subsample wss multiplied by the ratio of the volume of the sample to the volume of the subsample. No attempt was made to separate dead from live eggs.

Densities of copepod nauplii, the main microzooplankton component of the diet of young Pacific (Wailes 1935; Robinson 1988) and Atlantic (Checkley 1982; Cohen and Lough 1983; Fortier and Gagné 1990) herring larvae, were obtained from depth-specific 30-L water bottle samples at three stations inside Auke Bay, ABM, North Station (NOS), and midway between Coghlan and Spuhn islands (CSI), once very 7 d from April 5 to June 21, 1988, using techniques described by Paul et al. (1991). All sizes of nauplii were used, including those

<150 μm long, unlike Paul et al. (1991) who reported only densities of nauplii ≥ 150 μm long.

Samples of macrozooplankton and herring larvae were collected from five stations in Auke Bay from May to July 1988, ABM, NOS, CSI, off the breakwater of the Government dock (GOV), and in Auke Nu cove (ANC), with 3-m-long bongo nets each having a mouth diameter of 0.6 m and a hard plastic codend. Volume of water filtered was measured with a General Oceanics mechanical flowmeter placed in the mouth of one of the two nets. Nets with a mesh size of 165 μm were used for capturing macrozooplankton, and nets with mesh sizes of 333 and 505 μm were used for capturing herring larvae. The 333-μm mesh was used only during May when the population of herring larvae contained many newly hatched larvae because herring lavae <8 mm long are extruded through the 505-μm mesh (Colton et al. 1980; M. D. McGurk, unpubl. data). Nets were towed for about 10 min at about 1 $m \cdot s^{-1}$ in a double oblique pattern from the surface to a depth of 30 m and back to the surface. Contents of the codends were immediately preserved in either 5% formalin and seawater or 37% isopropyl alcohol and freshwater. Almost all of the plankton samples were taken during daylight between 9:00 and 20:00 local time.

Each formalin-preserved 165-μm sample was split several times and one subsample was completely identified and enumerated to the species level. Length and width were measured for several specimens of each taxon.

Prey density of herring larvae was derived by combining the mean density of copepod nauplii measured with the 30-L water bottle with densities of macrozooplankton prey measured with the 165-μm-mesh plankton nets. Copepod nauplii densities were pooled by depth and station to calculate a mean weekly nauplii density for the upper 30 m of Auke Bay.

It was not possible to define a prey field from Auke Bay herring larvae because too few of them had any gut contents to analyze. This was undoubtedly an artifact due to voiding of guts upon capture (Hay 1981). In the absence of information on the diet of herring larvae in southeast Alaska, we based the prey field on a series of reports on the diet of herring larvae and juveniles captured in the Strait of Georgia and Saanich Inlet in southern British Columbia, about 1300 km south of Auke Bay (Barraclough 1967a, 1967b, 1967c; Barraclough and Fulton 1967, 1968; Barraclough et al. 1968; Robinson et al. 1968a, 1968b; Robinson 1969 (hereafter called the BRF data)). This prey field is appropriate for herring larvae of southeastern Alaska because all of the species of copepods identified in Auke Bay in 1988 are either known to be common in southern British Columbia or have been reported from that area (Gardner and Szabo 1982). This is because the Alaska Current and the Alaska Coastal Current combine to mix the zooplankton of the continental shelf of the Gulf of Alaska, forming one large subarctic community of copepods (Cooney 1986). It is also appropriate because prey fields of herring larvae are similar worldwide. Over 70% of the prey of herring larvae and juveniles in southern British Columbia consisted of the eggs, nauplii, copepodites, and adult stages of calanoid copepods and cladocerans. The remaining prey included barnacle and euphausiid eggs, bivalve larvae, and adult amphipods and fish larvae. This prey field is similar to those reported for southern British Columbia (Wailes 1935; Robinson 1988) and for the northeast Atlantic Ocean (Cohen and Lough 1983; Fortier and Gagné 1990).

To define the prey field, it was necessary to know the expected maximum and minimum lengths and widths of prey for each length interval of herring larvae. The maximum and mean lengths of prey from the BRF data were regressed on the midpoint of 1-mm-wide herring length intervals:

$$\text{(1a)}\quad Y_{max} = -0.095 + 0.050L;\ n = 23,\ r^2 = 0.79,\ P < 0.01$$

$$\text{(1b)}\quad Y_{mean} = 0.160 + 0.015L;\ n = 23,\ r^2 = 0.36,\ P < 0.01$$

where Y_{max} = maximum prey length (millimetres), Y_{mean} = mean prey length (millimetres), and L = larval length (millimetres). Based on this analysis, the prey field of herring larvae in Auke Bay was defined as all taxa which had been found at least once before in a previous study of the diet of Pacific and Atlantic herring larvae and which were smaller in length than the maximum lengths predicted by equation (1) and smaller in width than the maximum prey widths reported by Checkley (1982). The final prey field included all species of calanoid copepods of the appropriate size plus cladocerans, mollusc veligers, polychaete larvae, and small fish eggs. It did not include copepod eggs because neither this study nor the concurrent APPRISE study used techniques that could accurately measure copepod egg density.

Prey concentration (milligrams dry weight per cubic metre) was calculated from prey density (numbers per cubic metre) using dry weight – length and dry weight – width relationships reported in the literature. Concentrations of all stages of copepods and cladocerans were calculated by first converting body width to wet weight using Pearre's (1980) equation for marine copepods. Wet weight in formalin was then converted to live wet weight by assuming a 10% loss in weight during storage, and live wet weights were converted to live dry weights by assuming an 80% water content. Concentration of mollusc veligers was calculated from data on mean live dry weight and mean length for larvae of the oyster, *Ostrea edulis* (Holland and Spencer 1973). Concentrations of polychaete trochophores and small fish eggs were calculated by assuming a spherical volume, a specific gravity of 1 $g \cdot cm^{-3}$, and a water content of 80%.

Densities of nonherring fish larva were measured at ABM station with a 1-m^2 Tucker trawl with a 505-μm mesh deployed in double oblique tows to a maximum depth of 30–35 m (Haldorson et al. 1989). ABM station was chosen because it was the primary station for measurement of physical and biological parameters of the concurrent APPRISE research program (Ziemann and Fulton-Bennett 1990). Volume filtered was measured with a flowmeter suspended in the trawl mouth. Five replicate samples were collected on one day each week from March 22 to June 21. All fish larvae were sorted, identified to species, and counted. Density was expressed as numbers per square metre of sea surface.

Juvenile herring were captured from August 14 to 25 with dip nets and beach seines. Juvenile sampling began on August 14 when schools first appeared inshore and stopped after August 25 when the fish grew too large to be caught with a dip net.

All herring larvae in the 333- and 505-μm bongo net samples, and all herring juveniles, were sorted from other plankton, counted, and measured for standard length to the nearest 0.1 mm. Lengths of formalin-preserved fish were corrected for shrinkage due to capture (McGurk 1985a) because this was a prerequisite for the use of McGurk's (1985b) morphometric condition factor. Larvae were classified into cohorts based on length frequency plots, using a computer program for separating mixtures of normal distributions (Tomlinson 1971). As a final check, lengths were plotted on day of year for each cohort,

and outliers, if present, were identified and reassigned to another cohort.

Specific growth in dry weight (percent per day) was calculated from mean length-at-date as

$$(2) \quad G_w = (100/t)\ln(W_{k+1}/W_k)$$

where W_k = mean dry weight (micrograms) at date k and t = time (days) between sampling dates. Mean dry weight was predicted from mean length-at-date using a weight–length regression for Auke Bay herring larvae.

To estimate specific growth rate from otolith increment number and width, two sagittal otoliths were removed from six fish from each alcohol-preserved bongo net sample and prepared for examination as described by Neilson and Geen (1980) and McGurk (1984a). A microscope with an attached video camera was used to measure the radius of each otolith, count the number of increments in each otolith, and measure the width of the outer four increments. Otolith radius was always measured along the longest axis of the otolith because herring otoliths become increasingly ovoid as they grow larger. Numbers of increments were counted along many radii (nucleus to outer edge) until the reader converged on a best estimate of average increment number for an otolith. The widths of the outer four increments were measured at two places on the otolith separated by 30–90°, and mean widths were used in all subsequent calculations of specific growth rate.

Specific rate of growth in dry weight (percent per day) was calculated from the average widths of the four outer increments with equation (2), except that W_k = dry weight (micrograms) of a fish with k increments and t = time (days) required to deposit one increment. Lengths were calculated from otolith radius using a regression of fish length on ln(radius). Radius was the distance from the center of an otolith to the furthest edge of an increment. The time for deposition of an increment was the reciprocal of the average slope of the regression of increment number on DOY. G_w was calculated using the mean radius and the mean increment width of the four outermost increments of each of the two sagittae or if one sagitta, if only one was available.

Herring larvae were classified as feeding or starving with a condition factor (CF) developed from herring larvae hatched from eggs collected from southwestern Vancouver Island and raised in enclosures (McGurk 1985b, 1989). The factor is based on five morphometric characters and dry weight:

$$(3) \quad \mathrm{CF} = 14.191 - 4.389\ln(L) + 2.184\ln(\mathrm{ABD}) + 2.197\ln(\mathrm{PBD}) - 12.331\ln(\mathrm{HW}) + 3.770\ln(\mathrm{ED}) + 0.419\ln(W)$$

where ABD = body depth (millimetres) at the anus, excluding the gut, PBD = body depth (millimetres) at the pectoral girdle, including the gut, HW = head width (millimetres), including the eyeballs, ED = posterior–anterior eye diameter (millimetres), and W = dry weight (micrograms). CF classifies larvae primarily on the width of the head and depth of the body relative to body size; both variables shrink with starvation and expand with feeding. We assume that CF is applicable to Auke Bay herring larvae, but this assumption has yet to be tested by comparing the length dependence of characters from different areas reared under known conditions. These experiments have not been performed for Auke Bay herring larvae. However, our assumption is supported by Robinson's (1988) finding that CF was highly correlated ($r^2 = -0.76$, $n = 29$, $P < 0.001$) with RNA–DNA ratio for wild Pacific herring larvae captured from the Strait of Georgia, British Columbia.

To calculate CF, 5–20 larvae were chosen randomly from each bongo net sample. Length of each fish was measured to the nearest 0.1 mm, and the other four morphometric characters were measured to the nearest 0.01 mm. Dry weight was measured by rinsing a larva in fresh water, drying it at 60°C for 24 h, and weighing it to the nearest 1 μg on an electrobalance. All dimensions were corrected for shrinkage or expansion caused by capture in towed nets (McGurk 1985a).

McGurk (1985b, 1989) calibrated CF so that feeding larvae had condition factors less than zero, and reversibly and irreversibly starving larvae had factors greater than zero. To calibrate CF for wild Auke Bay herring larvae, CF was regressed on ln(prey concentration); the intercept of this regression (the predicted value of CF when prey concentration was 1 $\mathrm{mg \cdot m^{-3}}$) was the critical value below which larvae were classified as feeding and above which larvae were classified as starving. This prey concentration of 1 $\mathrm{mg \cdot mm^{-3}}$ is close to the value of 2 $\mathrm{mg \cdot m^{-3}}$ at which the average instantaneous growth of laboratory-reared Atlantic herring larvae is zero (Kiørboe and Munk 1986).

Density of herring larvae was calculated as the number per square metre of sea surface. Larval density was corrected for underestimation due to avoidance of the net by herring larvae by multiplying density, distributed among 1-mm length intervals, by an avoidance correction factor and then summing corrected density across length intervals. We used McGurk's (1992) regression model of night–day catch ratios of herring larvae to estimate the appropriate avoidance correction factors for daytime catches, under the assumption that night–day catch ratios are caused by avoidance and that McGurk's (1992) regression model of night–day catch ratio is the best summary of the available data for herring larvae. Ratios ranged from 1.0 in larvae <9 mm long to 36.0 in larvae >25 mm long.

To measure mortality, it was necessary to adjust densities of herring larvae for dispersion out of the sampling area. Analysis of dispersion was based on an x–y coordinate system which was placed over Auke Bay with the origin arbitrarily set at the high tide level midway between Bay and Waydelich creeks at the spawning beach for cohorts 2 and 3 (Fig. 1). The x-axis was parallel to the north shore of the Bay and the y-axis extended offshore on a north–south axis parallel to the western shore of the Bay.

The x-coordinate of a centroid, or center of mass of a larval distribution, was

$$(4) \quad x_t = \sum N_{it} x_i / \sum N_{it}$$

where x_t = x-coordinate (metres) of centroid at age t (days), N_{it} = density of herring larvae (per square metre) at station i and age t, and x_i = x-coordinate (metres) of station i. The y-coordinate of a centroid was calculated similarly. Coordinates were calculated only for days for which data from a minimum of three different stations were available. Spatial variance of larval distribution in the x-axis, s_{xt}^2 (square metres), was

$$(5) \quad s_{xt}^2 = [\sum N_{it}(x_i - x_t)^2]/(\sum \mathrm{N}_t).$$

s_{yt}^2 was calculated similarly. Variances were calculated only for days for which data from at least three different stations were available.

Larval mortality and the number of newly hatched larvae were calculated from a population model that incorporated dispersion and mortality (Okubo 1980; Munk et al. 1986; Heath and MacLachlan 1987; McGurk 1989):

$$(6a) \quad N_{it} = \frac{M}{\pi(s_{xt}^2 \cdot s_{yt}^2)^{0.5}} \left[-\frac{(x_i - x_t)^2}{s_{xt}^2} - \frac{(y_i - y_t)^2}{s_{yt}^2} - Zt \right]$$

where M = number of herring larvae released into Auke Bay at the origin ($t = 0$, $x = 0$, $y = 0$) and Z = mortality (per day).

The model was fit to the larval density data by first rearranging equation (6a) so that all terms related to dispersion were placed on the left-hand side of the equation, i.e.

$$(6b)\quad n_t = N_{it}\pi(s_{xt}^2 \cdot s_{yt}^2)^{0.5} \exp\left[\frac{(x_i - x_t)^2}{s_{xt}^2} + \frac{(y_i - y_t)^2}{s_{yt}^2}\right] = M \exp(-Zt)$$

where n_t = number of herring larvae at age t. This number was transformed with natural logarithms and regressed on age, i.e.

$$(6c)\quad \ln(n_t) = \ln(M) - Zt.$$

Growth curves and the weight–length relationship were fit with nonlinear regression. All means were weighted by sample size divided by variance. Multiple linear regression with dummy variables for cohorts was used to calculate intercepts and slopes of regression of CF on DOY, otolith increment number on DOY, larval length on ln(otolith radius), x-coordinate of centroid on DOY, ln(larval density) on age, and ln(larval number) on age for each of the four cohorts. For example,

$$(7)\quad Y = b_0 + b_1X + b_2g_1 + b_3g_2 + b_4g_3 + b_5g_1X + b_6g_2X + b_7g_3X$$

where Y = dependent variable, X = independent variable, g_1 to g_4 are dummy variables with the values

Cohort	g_1	g_2	g_3	g_4
1	1	0	0	0
2	0	1	0	0
3	0	0	1	0
4	0	0	0	1

and b_0 to b_7 are regression coefficients. Significant variables were selected from the list of seven possible independent variables with stepwise regression (only $n - 1$ dummy variables were used to ensure a unique solution). r^2 was adjusted for the number of degrees of freedom in a regression model. Statistical power was calculated as described by Cohen (1988).

Results

Temperature and Salinity

Surface temperature of Auke Bay rose from a minimum of 4°C on March 14 (DOY = 74) to a maximum of 17°C on August 25 (DOY = 238). The rise was punctuated by transient maxima and minima caused by episodes of wind-driven mixing and advective exchange between Auke Bay and the surrounding fjord system. The water column was isothermal in mid- to late April (DOY = 106–121) with salinities ranging from 29.8 to 31.0‰ (Fig. 2). Advection of low-salinity surface water into Auke Bay in early May caused the water column to become thermally stratified and caused rapid increases in depth of the isohaline lines. A sudden increase in mixing energy in the last half of June (DOY = 167–182) destroyed thermal stratification and caused a decrease in depth of the isohaline lines. Stratification was reestablished in early July.

Prey Concentration

The taxonomic composition and temporal changes in abundance and biomass of zooplankton in Auke Bay in May–June 1988 were described in detail by Coyle et al. (1990), Coyle and Pual (1990a, 1990b), and Paul et al. (1990a, 1990b, 1991). We note only that in 1988 the density of all sizes of copepod nauplii was nearly always highest at 5 m and decreased with depth and that herring larvae entered the water column during the period of highest densities of copepod nauplii (Fig. 3).

Mean weekly concentration of prey for herring larvae in Auke Bay was strongly influenced by advective exchange of water between Auke Bay and Lynn Canal. Although mean prey concentration for herring larvae ≤10 mm long was roughly constant at about 20 $mg \cdot m^{-3}$ from May to June, prey concentration for larger herring larvae exhibited two maxima, one on May 27 (DOY = 148) and a second on June 20 (DOY = 172), separated by a minimum on June 8 (DOY = 160) (Fig. 3). This pattern coincided with the timing of advective exchange between Auke Bay and outside waters. The first maximum occurred at the same time as the first advection of outside water into Auke Bay, the minimum was reached during the middle of the following period of stratification, and the second maximum occurred when this stratification was destroyed by the first turbulence event. This pattern reflects the fact that large calanoid copepods, which constituted most of the two prey maxima, are usually absent from shallow Alaska bays in spring unless advected in because their copepodid and adult stages are vertical migrators and so require more than the 40 m of water that is found in Auke Bay (Coyle et al. 1990). Smaller copepods that were the prey of small herring larvae were presumably also advected in and out of the Bay, but because their density outside Auke Bay was similar to their density inside the Bay (Paul et al. 1991), their concentration was not altered by advective processes.

These prey densities and concentrations are high relative to the levels required for successful growth of herring larvae. The average depth-integrated density of copepod nauplii of $27 \cdot L^{-1}$ in early May is close to the level of $30 \cdot L^{-1}$ that saturates feeding of laboratory-reared Atlantic herring larvae (Kiørboe and Munk 1986). The mean weekly prey concentrations of ≥20 $mg \cdot m^{-3}$ for all length classes of herring larvae are sufficient to support growth in weight of at least $4\% \cdot d^{-1}$, and they are an order of magnitude higher than the prey concentration of 2 $mg \cdot m^{-3}$ that induces starvation in laboratory-reared herring larvae (Kiørboe and Munk 1986).

Distribution and Density of Herring Eggs

Four spawnings of herring occurred in or near Auke Bay in 1988, but only the eggs of cohorts 2 and 3 were discovered and mapped. On May 5–7 the parents of the second cohort were observed spawning on the northeastern shore of the Bay in the intertidal zone of a beach between Waydelich Creek on the west and Bay Creek on the east (Fig. 1). Eggs were laid on narrow strips of *Fucus* and *Desmarestia* in the upper intertidal zone. Almost all eggs were laid above mean lower low water, far higher than is usual for eggs from the main spawning group in Lynn Canal. The strips covered a total length of about 250 m. A SCUBA survey on May 18 found no eggs in the subtidal zone and no vegetation on which eggs could have been laid (M. Stekoll, Juneau Center for Fisheries and Ocean Sciences, University of Alaska, 11120 Glacier Highway, Juneau, AK 99801, pers. comm.). Spawning by parents of the third cohort was not observed, but it must have occurred shortly after May

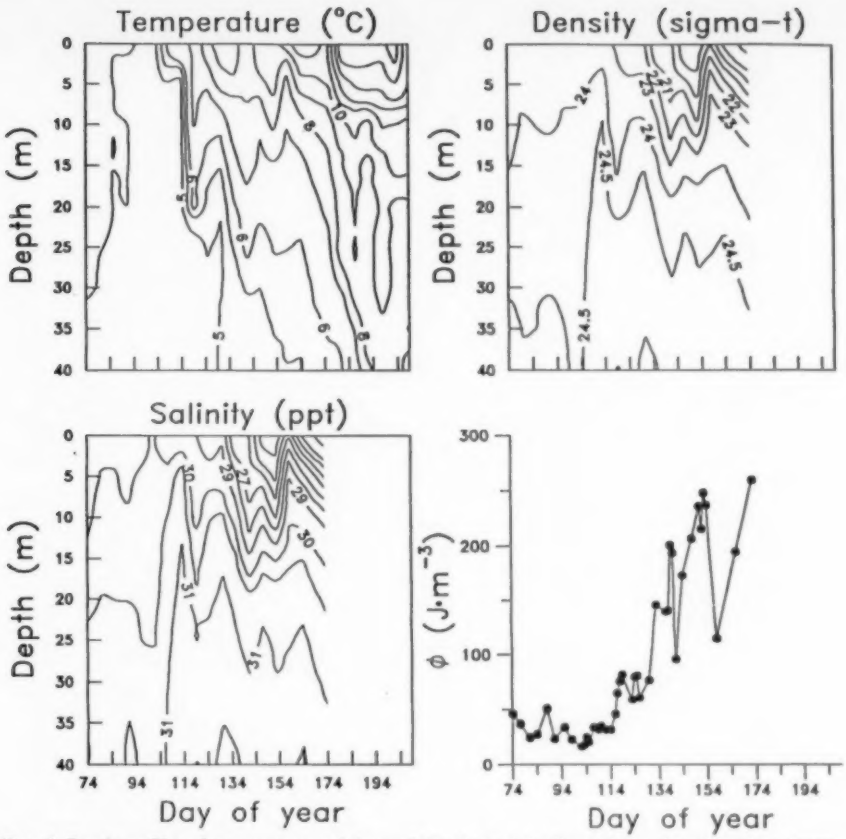

FIG. 2. Depth profiles of temperature, salinity, and density in Auke Bay, Alaska, from March to August 1988. Simpson's (1981) stratification parameter, ϕ, is defined as the amount of work needed to fully mix the water column.

29 because eggs were first observed on June 5. These eggs were laid on the same patches of vegetation that held the eggs of the second cohort.

The distributions of eggs of the second and third cohorts on the beaches were mapped on May 18 and June 6, respectively. Geometric mean density of cohort 2 eggs was about half that of cohort 3 eggs, but cohort 2 eggs covered more than four times the area covered by cohort 3 eggs, so the total number of cohort 2 eggs was twice as high as the total number of cohort 3 eggs (Table 1).

These numbers of eggs illustrate the "trace" nature of herring spawning in Auke Bay; they are equivalent to only 10.4 t of spawners for cohort 2 and 4.5 t for cohort 3, assuming a specific fecundity of 200 eggs·g wet female tissue^{-1} and a 1:1 sex ratio (Hay 1985). These are minimum spawner biomasses because some eggs were eaten by predators or dislodged from the spawning beds over the 9- to 13-d incubation period. The total spawning biomass of 14.9 t was only about 1% of the biomass measured off the primary spawning sites north of Auke Bay (D. Ingledue, Alaska Department of Fish and Game, Commercial Fisheries Division, P.O. Box 240020, Douglas, AK 99824-0020, pers comm.).

Spawning and Hatching Dates

Length frequency plots indicate that four cohorts of herring larvae hatched in Auke Bay in 1988 (Fig. 4). Their dates of hatch were estimated with three independent methods (Table 2). Since the three methods were independent of each other, their mean was chosen as the best estimate of day of hatch. Hatching of herring eggs occurred over a period of 52 d. The average number of days between hatchings was 17 d (SD = 4), similar to cohort spacings reported by Lambert (1984) and McGurk (1989), and the average number of days between spawnings was 20 d (SD = 5).

Growth

Growth in length of three of the four cohorts was well described by a modified one-cycle Gompertz model (Zweifel and Lasker 1976):

$$(8)\quad L_D = L_0\exp[(A_0/a)\{1 - \exp[-a(\mathrm{DOY} - \mathrm{DOY}_0)]\}]$$

where L_D = length (millimetres) at DOY, L_0 = length (millimetres) at DOY_0, the DOY of hatch, A_0 = rate of growth (per day) at DOY_0, and a = rate of change (per day) of A_0 with age (Table 3; Fig. 5A). Equation (8) differs from the usual Gompertz growth model in that L_0 was fixed at 8.8 mm so that DOY_0 could be estimated as a third free parameter. Information about date at hatch was judged to be more important than information about L_0. Since only four of the 97 samples had one or more yolk sac larvae, we obtained an estimate of L_0 from a regression of mean length on fraction of yolk sac larvae for samples of herring larvae collected in Prince William Sound, Alaska, using

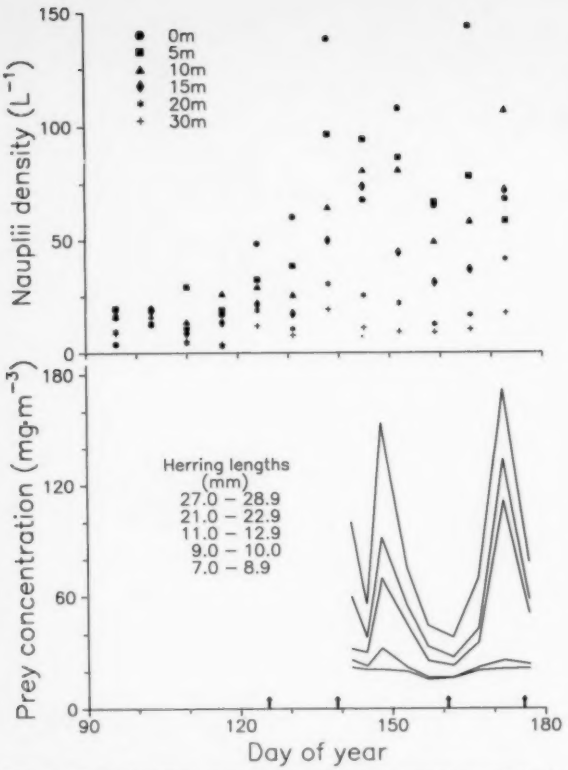

FIG. 3. Mean densities of all copepod nauplii (<150 to >350 μm long) at six depths and mean prey concentrations for five length classes of herring larvae in Auke Bay, Alaska, from April to June 1988. Arrows mark dates at hatch for four cohorts of herring larvae.

the same techniques employed in this study (McGurk et al. 1990).

Equation (8) provided a good fit to the mean lengths at date of cohorts 1 and 3 because their growth rate decreased smoothly with date and age, and of cohort 4 because its growth was essentially linear due to low sample size. However, equation (8) provided a poor fit to the mean lengths at date of cohort 2, as judged by visual means, because growth was more complex for this cohort; it was initially low, increased in the mid- and late larval stages, and then decreased during the transition from the larval to juvenile stages. A two-cycle version of equation (8) would be necessary to describe this growth pattern. The high r^2 for the fit of equation (8) to cohort 2 was due to the fact that 88% of the larvae of this cohort were captured before the second cycle of growth began after DOY 170.

This difference in growth between cohorts was also evident in plots of G_w calculated from growth curves and a weight–length regression (Fig. 5B). G_w followed a dome-shaped pattern with age for all four cohorts, but cohort 2 had a lower rate of initial increase in G_w and a lower maximum G_w than the other three cohorts.

G_w estimated from otolith increment width also exhibited a dome-shaped relationship with DOY, but it did not show any among-cohort differences (Fig. 6C). To test for among-cohort differences in otolith G_w we compared the residuals of a simple regression model among cohorts. The model was

$$(9a) \quad G_w = \alpha t \exp(-\beta t)$$

where α (percent per day squared) is the rate of increase of otolith G_w with age t (days) and β (per day) is the rate of decrease in otolith G_w with age. The model was linearized by rearranging terms and transforming with natural logarithms, and then it was regressed on age (Fig. 6C):

$$(9b) \quad \ln(G_w/t) = 0.03565 - 0.03965t;\ n = 132,\ r^2 = 0.626,\ P < 0.01.$$

According to this model, average otolith G_w rose from 4.2%·d^{-1} at an age of 5 d to a maximum of 9.6%·d^{-1} at 28 d and then fell to 6.7%·d^{-1} at 50 d. These otolith G_w are similar to those estimated from length-at-age data, with the exception that the residuals of the regression were not significantly ($P > 0.05$) different among cohorts.

Condition

A multiple regression of CF on date and dummy variables for cohorts showed that condition improved with age in all four cohorts of herring larvae (Fig. 6D) but that mean CF of small larvae of cohort 2 was significantly lower than in the other three cohorts and that condition of cohort 2 improved with age at a faster rate than the rates of the other three cohorts.

The value of CF separating starved from fed larvae was estimated by regressing CF on ln(prey concentration); the regression intercept of 2.081 (SE = 1.627) was greater ($P < 0.001$) than zero (Fig. 7B). However, the low r^2 of this regression indicates that there is substantial uncertainty associated with the intercept. Between 8 and 35% of first-feeding herring larvae had CF values greater than this critical value and so were classified as starving (Fig. 8A).

All starving larvae were captured between DOY 136 and 162. Cohort 2 had the longest period of continuous incidence of starving larvae (Fig. 8A): 21 d from DOY 136 to 157. Because that was a period of high prey concentration for all size classes

TABLE 1. Number of herring eggs laid on the Auke Bay spawning beach. DOY is date of egg survey. Parentheses enclose the range of egg densities ($n = 5$). Number of eggs is the product of mean egg density and area of spawn. Number of newly hatched larvae was estimated from the regression equation for larval numbers shown in Fig. 10.

Cohort	DOY	Geometric mean egg density (m^{-2})	Spawn area (m^2)	Measured number of eggs	Predicted number of newly hatched larvae
2	139	352 040 (80 400–994 500)	2949	10.38×10^8	4.95×10^8
3	157	681 806 (182 800–3 166,900)	662	4.51×10^8	4.95×10^8

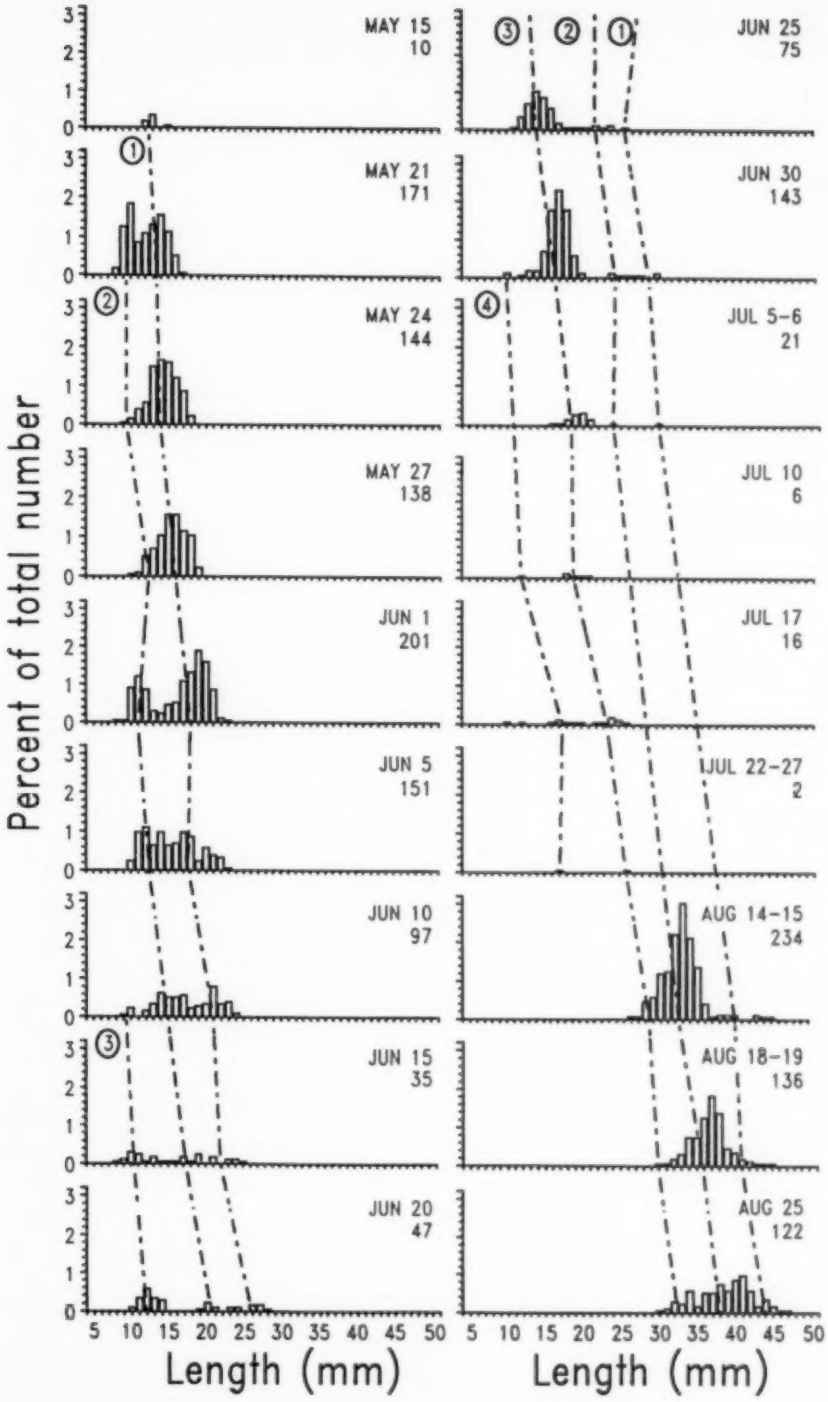

FIG. 4. Length frequencies of four cohorts of herring larvae from Auke Bay, Alaska. Broken lines connect mean lengths at date. Numbers beneath dates are the number of fish captured on that date.

TABLE 2. DOY of hatching and spawning for four cohorts of Auke Bay herring larvae. Hatch date was the mean for two or three independent methods. Day of spawn was the difference between the mean day of hatch and incubation time.

Method	Cohort 1	2	3	4
Growth curve[a]	128	142	164	179
Fraction yolk sac[b]	—	137	162	—
Otolith increments[c]	123	138	157	173
Mean	125 (May 4)	139 (May 18)	161 (June 9)	176 (June 24)
Incubation time (d)[d]	19	13	11	11
Day of spawn	106 (Apr. 15)	126 (May 5)	150 (May 29)	165 (June 13)

[a]DOY_0 (Table 3).

[b]Age (d) = $40.9T^{-0.84}(1 - f)$ where T = average temperature (°C) of the upper 30 m measured on the same day as the sample was collected and f = fraction of yolk sac larvae in the sample. Data from Alderdice and Velsen (1971) were used to calculate a regression of duration of yolk on temperature ($n = 12$, $r^2 = 0.67$, $P = 0.001$). Mean hatch date was the mean difference between date of capture and age at capture, weighted by the density of herring larvae measured on that date.

[c]Based on intercepts of the multiple regression of increment number on DOY using dummy variables for cohorts. The rate of increment deposition was a free parameter estimated by regression — it was not assumed to be daily. Since the first visible otolith increment in herring is deposited after the exhaustion of the yolk (McGurk 1984a; Campana et al. 1987), the second step was to calculate, for each cohort, the duration of the yolk sac stage and subtract it from the DOY of zero increment to obtain DOY_0.

[d]Estimated from mean surface water temperature using Alderdice and Velsen's (1971) equation (9).

TABLE 3. Parameters of modified Gompertz growth models (equation (8)) for four cohorts of Pacific herring larvae from Auke Bay, Alaska, during May–August 1988.

Cohort	A_0 (d^{-1})	SE	a (d^{-1})	SE	DOY_0	SE	r^2	n
1	0.0344	0.0012	0.0191	0.0009	128	1	0.946	656
2	0.0287	0.0006	0.0153	0.0006	142	1	0.978	718
3	0.0431	0.0006	0.0295	0.0007	164	1	0.963	362
4	0.0425	0.0348	0.0314	0.0742	179	4	0.800	11

of herring larvae, ancillary factors must have prevented cohort 2 larvae from exploiting all available prey during that period.

Mortality

To calculate mortality, we first converted larval densities to larval numbers by adjusting for the effects of dispersion. An absence of offshore advection was shown by a lack of correlations ($P > 0.05$) between the y-coordinate of the centroids and age, DOY, or dummy variables for cohorts 1–4 (Fig. 9). The mean y-coordinate was not significantly ($P > 0.05$) different from the y-coordinate for ABM station (Table 4).

However, from May 22 to July 19 the x-coordinate of the centroids of larval herring density moved westwards across Auke Bay at an average rate of 20 $m \cdot d^{-1}$ (Fig. 9). A single regression of x-coordinate on DOY fit the data for all four cohorts. The x-coordinate was also correlated with age, but the relationship explained 14% less variance than DOY.

There were no significant correlations between the spatial variances in the x- and y-axes and age, DOY, or dummy variables for cohorts (Fig. 9). Mean s_{xt}^2 was eight times lower than mean s_{yt}^2 (Table 4), which meant that patches of herring larvae were elongated along the y-axis in a pattern similar to the north–south bathymetric orientation of Auke Bay. This suggests that herring larvae dispersed through the main channel east of Coghlan Island.

Density of cohorts 1 and 2 decreased at a constant exponential rate, but the catch curves of cohorts 3 and 4 were dome-shaped, indicating that larvae were not fully recruited to the plankton net until an age of at least 20 d (Fig. 10). Therefore, densities of cohorts 3 and 4 for ages <20 d were excluded from calculations of rate of loss of density with age. Multiple regression on ln(density) on age with dummy variables for cohorts showed that larval density was lost at a rate of $0.0452 \cdot d^{-1}$ (SE = 0.0101) for cohorts 2, 3, and 4 and at a rate of $0.0824 \cdot d^{-1}$ (SE = 0.0132) for cohort 1 (Fig. 10) and that larval density at hatch of cohort 1 was greater than for the other three cohorts and at hatch of cohort 4 was lower than for the other three cohorts.

These loss rates included losses due to dispersal of larvae outside of the sampling area as well as mortality. The effect of dispersal was removed by, first, converting larval density to larval number using equation (6b) and the parameters of Table 4 and then ln-transforming this number and regressing it against age (Fig. 10). Numbers of larvae for cohorts 1, 2, and 4 decayed with age at a constant exponential rate, but the catch curve of cohort 3 was still dome-shaped, indicating that dispersal may not have been responsible for the delayed age of recruitment in that cohort. Numbers of cohort 3 larvae with ages <20 d were excluded from subsequent analyses.

All four cohorts had the same average mortality of $0.0631 \cdot d^{-1}$ (SE = 0.0152); cohort 1 had greater initial number

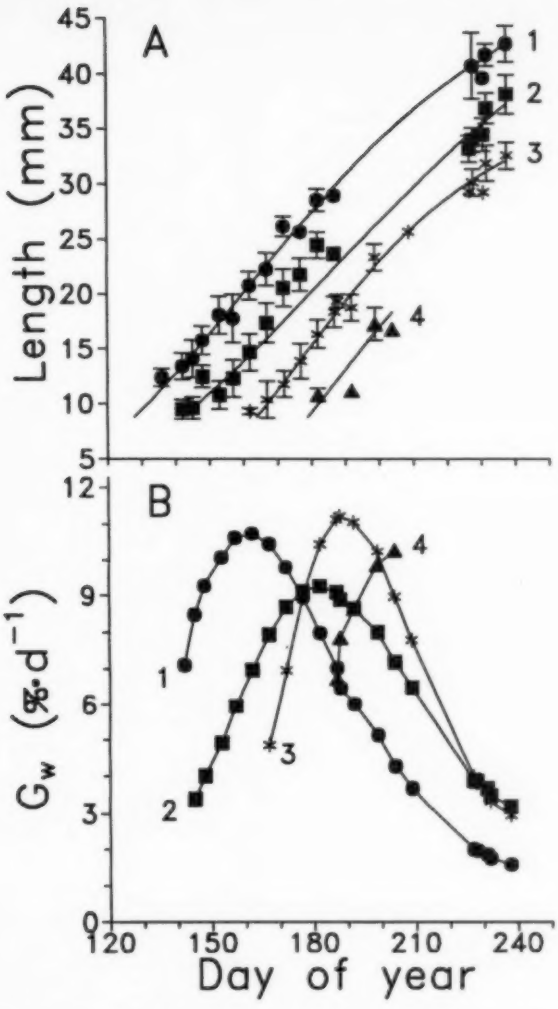

FIG. 5. Growth of four cohorts of herring larvae and juveniles from Auke Bay, Alaska. (A) Mean (± 1SD) length at date. Growth curves are modified Gompertz models (equation 8)). The intercepts of the curves with a length of 8.8 mm are estimates of the average dates of hatch or DOY_0. (B) Instantaneous growth in weight of herring larvae calculated from the mean lengths at date predicted by equation (8) and a logistic weight–length regression fit to the combined data of cohorts 1–4: $\ln(W) = 4.653 + [8.536/1 + \exp\{-2.968[\ln(L) - 3.208]\}]$; $n = 372$, $r^2 = 0.99$, $P < 0.001$.

and cohort 4 had lower initial number than the other three cohorts (Fig. 10). The accuracy of this estimate of mortality is supported by the fact that the number of eggs counted for cohorts 2 and 3 were not significantly ($P > 0.05$) different from the average number of newly hatched larvae for both-cohorts, 4.95×10^8. In fact, the percentage of late-stage eggs that hatched viable larvae, 48–110%, brackets the average percentage of 60–70% that is expected for low-density spawnings of Pacific herring eggs (Hourston et al. 1984).

However, the statistical power of the multiple regression of ln(numbers) on age was relatively low, ranging from 0.19 for cohort 2 to 0.32 for cohort 1. This means that the probability of incorrectly accepting the null hypothesis of no differences in mortality between cohorts ranged from 0.68 to 0.81 — too high to be confident that there were no differences between cohorts.

Discussion

Timing of Hatching

Production of copepod nauplii in Auke Bay in sufficient densities to support successful feeding and growth of young herring larvae, i.e. ≥ 15–20 nauplii·L^{-1}, occurs within a period of about 1–2 mo (Paul et al. 1991). The fact that herring larvae entered the water within this time window suggests that food, or the lack of it, has the potential to constrain larval herring population dynamics in Auke Bay.

The limited data available suggests that an ecologically important mismatch between timing of herring larvae hatch and timing of the copepod maximum in Auke Bay is possible, but unlikely. The date of maximum density of herring larvae does not vary by more than about 2 wk, but the date of maximum density of copepod nauplii varies by at least 4 wk (Table 5). However, the likelihood of a complete temporal mismatch between herring larvae and their prey in Auke Bay is low because the spawning strategy of herring means that at least one cohort will hatch into the period of maximum prey concentration.

The reason for occasional asynchronous development of herring larvae and their prey is that the two sets of organisms are responding to slightly different sets of environmental variables. Timing of Pacific herring spawning in areas free of ice is a function of temperature: the higher the temperature, the earlier the time of spawning (Hay 1985; Ware and Tanasichuk 1989). This trend continues through to hatching because egg incubation time is strongly dependent on temperature (Alderdice and Velsen 1971). In contrast, the timing of the herbivorous copepod maximum depends not only on temperature, which controls generation time (Coyle et al. 1990), but also on the abundance of overwintering reproductively active females in April (Paul et al. 1990b). This is because female herbivorous copepods depend on the spring bloom of phytoplankton for energy to produce eggs. If female copepod abundance is high before the bloom due to the presence of overwinterers, then the peaks of egg and nauplii production will be reached early — perhaps within one generation. A copepod generation is about 60 d. However, if female copepod abundance is low before the bloom, then the copepod maximum will be delayed one or two generations as the female abundance increases due to recuitment of females born after the start of the spring bloom.

Events in 1988 did not support a close temperature-mediated link between timing of herring and their copepod prey. Surface water temperature of Auke Bay was higher in late April and early May 1988 than in any other of the earlier years (Table 5), so hatching of herring in 1988 was at least 10 d earlier than in other years. However, peak nauplii densities were not earlier in 1988 than in previous years; instead the date of the first peak mean nauplii density in 1988 was the same as the date in 1986 and about 28 d later than the peak in 1987 (Paul et al. 1991).

We conclude the temperature-mediated synchrony between herring and their prey in Auke Bay is limited to periods of weeks or months rather than days. As Lambert (1984) and Lambert and Ware (1984) suggested, this may be one reason why both

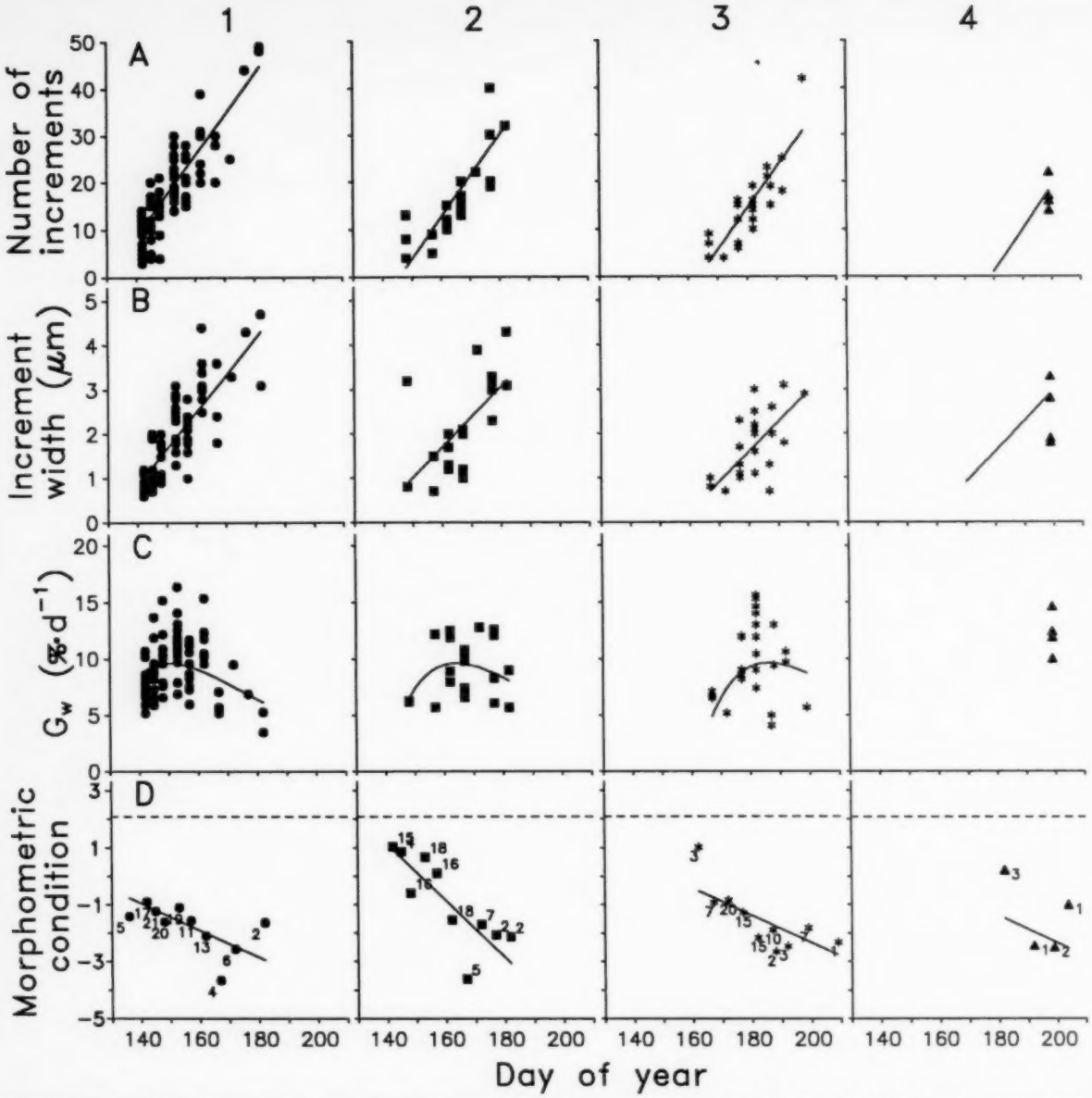

FIG. 6. Growth and condition of four cohorts of herring larvae from Auke Bay, Alaska, plotted against DOY. (A) Number of increments in the sagittal otoliths. Solid lines are the number predicted from the following regression: number = $-113.6 + 0.87\text{DOY} - 12.9g_2 - 28.8g_3 - 41.6g_4$; $n = 132$, $r^2 = 0.725$, $P < 0.001$. The x-axis intercepts are the dates at which yolk sacs were absorbed, about 5 d after hatching. (B) Mean width of increments in the otoliths. Solid lines are the widths predicted from the following regression: width = $-10.72 + 0.0684\text{DOY} + 1.474g_2 + 0.0141g_1\text{DOY}$; $n = 133$, $r^2 = 0.595$, $P < 0.001$. (C) Specific growth rate, G_w, estimated from otolith increment widths, a regression between fish length and otolith radius for the combined data of cohorts 1–4 ($L = 45.02 - 7.92\ln(\text{radius})$; $n = 134$, $r^2 = 0.87$, $P < 0.001$) and the weight–length regression of Fig. 5B. The solid lines are a regression of G_w on age for the pooled data of cohorts 1–4. (D) Mean morphometric condition. Numbers are sample sizes. Solid lines are condition factor predicted by the regression $\text{CF} = 7.196 - 0.0477\text{DOY} - 1.470g_1 + 7.970g_2 - 0.0524g_2\text{DOY}$; $n = 311$, $r^2 = 0.253$, $P < 0.001$. The broken line is the critical value above which larvae were classified as starving.

Pacific and Atlantic herring have a complex cohort structure. To counteract environmental variability they have evolved a "bet-hedging" strategy of spreading reproductive effort over time by spawning in three to six waves, each separated by 15–25 d.

Growth

Average growth in length of Auke Bay herring larvae in 1988 of about 0.3 mm·d^{-1} is similar to that reported by Jones (1978), and it falls within the upper part of the range reported for wild

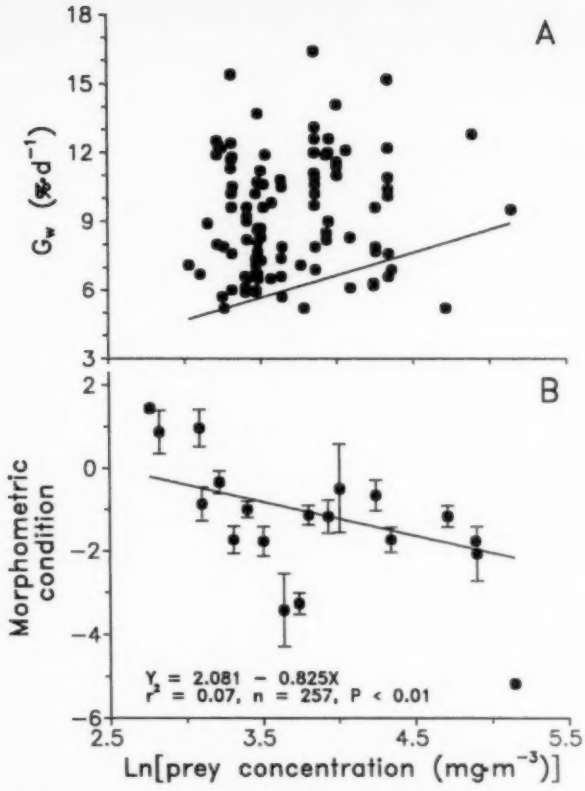

FIG. 7. (A) Plot of otolith G_w against prey concentration. Line is G_w predicted by Kiørboe and Munk's (1986) equation for laboratory-reared Atlantic herring larvae. (B) Regression of mean (±1 SE) morphometric condition on prey concentration. The intercept of the regression is the critical CF above which larvae are classified as starved.

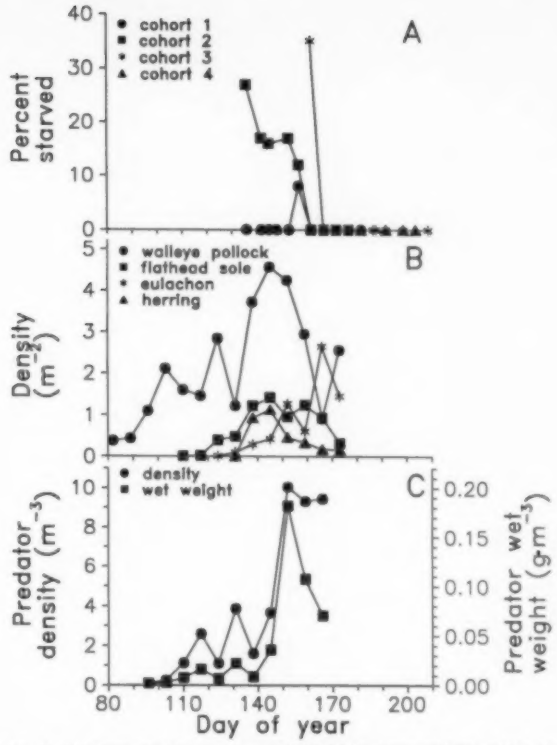

FIG. 8. (A) Percent of starvation with DOY for three cohorts of herring larvae, (B) catch curves of mean density at ABM station in 1988 of four species of fish larvae that spawned in Auke Bay during the herbivorous copepod maximum, and (C) density and wet weight of invertebrate predators on copepods.

herring larvae by McGurk (1984b). The range of mean G_w for Auke Bay herring larvae was of similar magnitude to that reported by Munk et al. (1991) for Atlantic herring larvae of the North Sea.

The findings of our analysis of growth were that (1) G_w calculated from length frequency analysis identified slow- and fast-growing cohorts, (2) G_w derived from otolith increments did not, and consequently otolith G_w was higher than predicted by laboratory-feeding experiments, and (3) there was no significant correlation between G_w and prey concentration.

The main reason for the absence of between-cohort differences in otolith G_w is that young first-feeding larvae and poorly conditioned larvae do not always deposit increments wide enough to be detected by optical microscopy. The lowest growth rate that could be estimated with this method, 4%·d^{-1}, was set by the smallest measurable increment width, 0.6 μm. This is why there was no significant ($P > 0.05$) regression of otolith G_w on ln(prey concentration) (Fig. 7A).

To understand this phenomenon, we examined the calculation of otolith G_w. Multiple regression showed that there was only one average rate of increment deposition, 0.87·d^{-1} (SE = 0.05) (Fig. 6A), for all four cohorts and that it was significantly ($0.001 < P < 0.01$) lower than 1.0·d^{-1}, a finding that was previously reported for herring larvae by Geffen (1982), McGurk (1984a), and Campana et al. (1987). The latter authors argued that less than daily increment deposition in herring larvae was due to undercounting of daily increments because optical microscopes cannot, in theory, resolve increments <1 μm wide. Their hypothesis predicts that average increment width should be about 1 μm in the smallest otoliths. To test this hypothesis, we regressed mean increment width of the outer four increments on DOY and dummy variables for cohorts (Fig. 6B). This regression supported the hypothesis by showing that average increment width in the smallest otoliths was about 1 μm and that this occurred 10–17 d after hatch and 2–9 d after exhaustion of yolk, which would be expected if slow growth during first-feeding produced some increments with widths too narrow to be detected by optical microscopy.

The hypothesis of increment undercounting also predicts that mean otolith G_w overestimates the true population mean G_w. This was supported by the observation that mean otolith G_w of herring larvae in Auke Bay was 3.1%·d^{-1} greater than G_w predicted by Kiørboe and Munk's (1986) regression of G_w on prey concentration for 1- to 3-wk-old Atlantic herring larvae reared on copepod nauplii (Fig. 7A).

The absence of significant relationships between otolith G_w and prey concentration and temperature may have been due to larvae feeding in essentially ad libitum conditions, so no gradient of feeding conditions occurred or, at least, no gradient sufficiently large to be detected by our methods. A lack of contrast in the environmental data may also have been responsible. Although mean prey concentrations from May 21 to June 25 ranged from 20 to 110 mg dry weight·m^{-3}, the semilogarithmic nature of Kiørboe and Munk's (1986) G_w–prey relation-

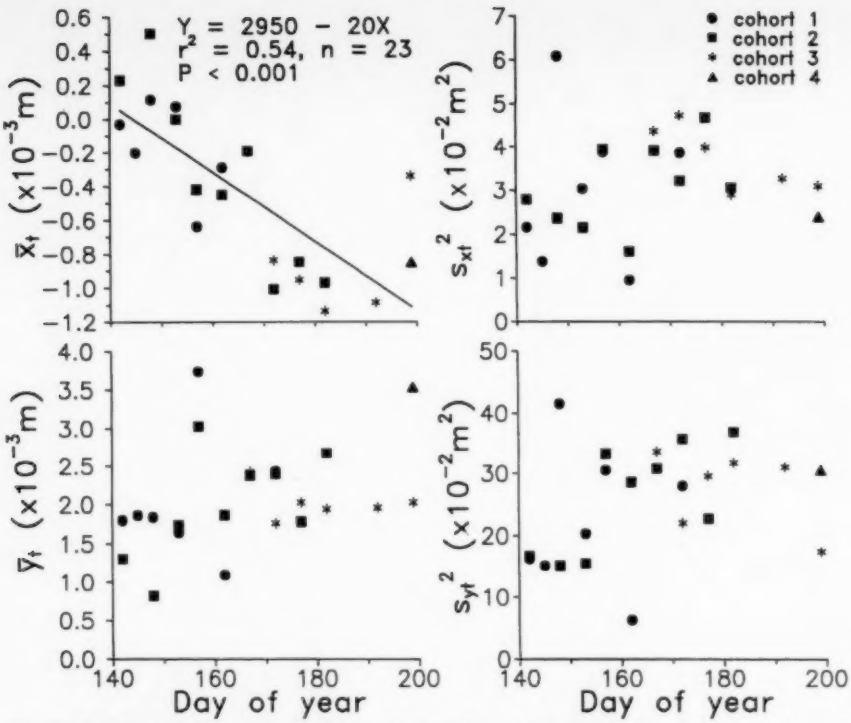

FIG. 9. Spatial distribution of four cohorts of herring larvae from Auke Bay, Alaska: regression of x-coordinate of centroids on day of year, plot of y-coordinate of centroids on day of year, plot of spatial variance of larval density along the x-axis on day of year, and plot of spatial variance of larval density along the y-axis on day of year.

TABLE 4. Parameters used in equation (6b) to convert larval densities to larval numbers. Sample number was 23.

Parameter	Units	Value	SD
x_t	m	2950–20DOY	
y_t	m	2091	691
s_{xt}^2	m^2	3.21×10^5	1.20×10^5
s_{yt}^2	m^2	2.56×10^6	8.92×10^5

ship means that the predicted increase in G_w over this range of prey concentrations is only $3.4\%{\cdot}d^{-1}$. Also, mean temperatures of the upper 20 m of the later column over the same time period fell within a narrow range of only 7.2–8.2°C.

Condition

By hatching into the spring copepod maximum in1988, herring larvae in Auke Bay entered a food-rich environment that should have promoted fast growth, high condition, and low mortality. Paul et al. (1991) reached such a conclusion for cohorts of walleye pollock and flathead sole larvae in Auke Bay in 1987, 1988, and 1989. Densities of copepod nauplii 150–350 μm long in May of all three years were never low enough to cause mass starvation in walleye pollock and flathead sole larvae.

However, despite this apparently rich food source, 8–35% of first-feeding herring larvae were classified as reversibly or irreversibly starving. This range of percentages is similar to that reported by Robinson and Ware (1988) for herring larvae from the Strait of Georgia, British Columbia, that were analyzed with RNA–DNA ratios. For our purposes the exact range of percent starvation is less important than the timing of its incidence and the way it was distributed among cohorts.

One factor that may have prevented cohort 2 larvae from exploiting available prey was density-dependent competition for food with other herring larvae. We tested this hypothesis by comparing the mortality of prey due to herring predation with the total mortality of prey. Following Kiørboe et al. (1988), we assumed that a 12-mm-long herring larvae cleared 1.5 L of water of food particles every daylight hour and that this clearance rate increased directly with the cube of larval length. Thus, mortality of prey (per day) = clearance (litres per hour) × pooled density (per cubic metre) of herring larvae of all four cohorts × feeding period ($12\ h{\cdot}d^{-1}$) = $0.08{\cdot}d^{-1}$ in mid-May to $0.05{\cdot}d^{-1}$ in mid-June. This was one half to one third of the total mortality of about $0.15{\cdot}d^{-1}$ estimated for a copepod nauplius with an average dry weight of 1.6 μg using Peterson and Wroblewski's (1984) regression model of weight-dependent mortality in the marine pelagic ecosystem.

Since Peterson and Wroblewski's (1984) model is only applicable to average conditions in the pelagic ecosystem, the hypothesis was also tested by comparing mortality of prey due to herring larvae with maximum production of prey. Paul et al. (1990b) reported that the average production of *Pseudocalanus* spp. eggs in Auke Bay in 1988 was 2.6 $eggs{\cdot}female^{-1}{\cdot}d^{-1}$, which is equivalent to a specific production of $0.96{\cdot}d^{-1}$ (= ln(2.6)). This is at least 12 times greater than the mortality rate due to herring predation. Additional nauplii would be produced by the other copepod species present, so we conclude

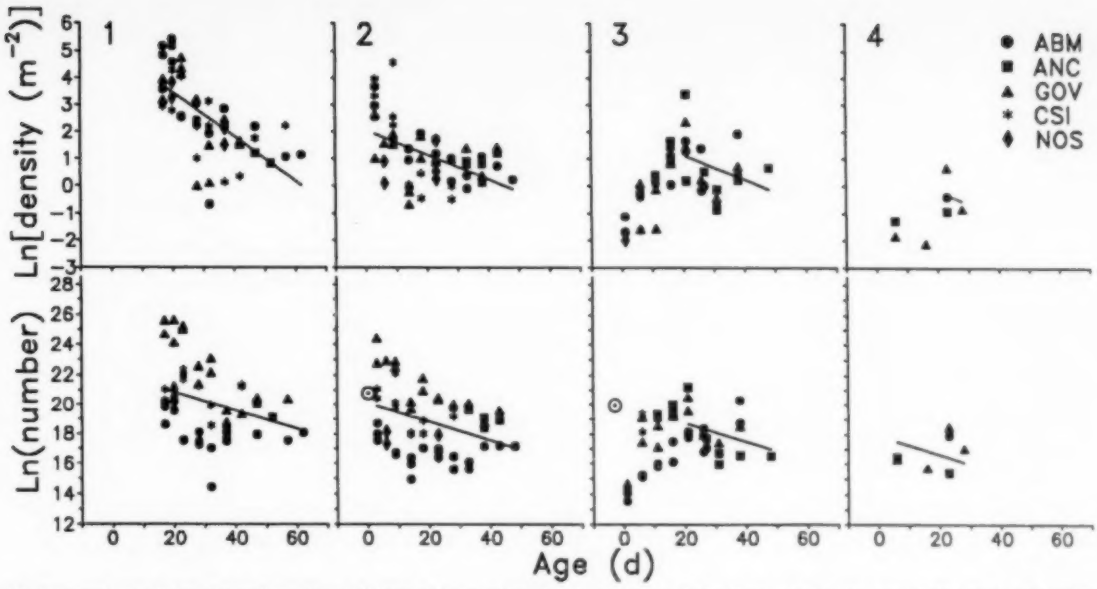

FIG. 10. Regressions of density and numbers of four cohorts of herring larvae from Auke Bay, Alaska, on age. Solid lines of densities are predicted from the regression $\ln(N) = 2.058 - 0.0452t + 3.082g_1 - 1.311g_4 - 0.0372g_1t$; $n = 129$, $r^2 = 0.520$, $P < 0.0001$. Solid lines of numbers are predicted from the regression $\ln(n) = 20.0205 - 0.0631t + 2.096g_1 - 2.122g_4$; $n = 132$, $r^2 = 0.242$, $P < 0.0001$. Open symbols are numbers of herring eggs counted on the spawning beach of Auke Bay.

TABLE 5. Dates of maximum density of Auke Bay herring larvae (H) and copepod nauplii (N) compared with mean surface water temperatures. Dashes indicate no data available.

	DOY of maximum density		Mean (± 1 SE) surface temperature (°C)				
Year	H[a]	N[b]	Apr. 1–14	Apr. 15–30	May 1–14	May 15–31	Source
1988	142	144	4.68 (0.07)	6.91 (0.18)	8.84 (0.37)	9.95 (0.23)	This study
1987	154	113	5.53 (0.07)	5.39 (0.20)	7.60 (0.50)	8.34 (0.38)	Haldorson et al. 1988
1986	154	144	3.45 (0.10)	4.25 (0.18)	7.56 (0.23)	8.19 (0.37)	Haldorson et al. 1987
1976	153	—	2.90 (0.57)	5.30 (0.92)	5.20 (0.07)	6.50 (0.35)	Jones 1978
1975	157	—	3.10 (0.78)	6.40 (0.80)	6.30 (0.42)	8.60 (0.64)	Jones 1978

[a]Date of maximum density is an index of hatching date of the first and most abundant cohort.
[b]Paul et al. (1991).

that the hypothesis of density-dependent competition for food between herring larvae is not supported — they were simply too dilute to affect prey concentrations.

A second possible cause of starvation was competition for food with other species of fish larvae and with invertebrate predators of copepods. We lack the detailed information on clearance rates that are necessary to test this hypothesis using the methods used for testing the density-dependent hypothesis, so we tested it by comparing the temporal trend of starvation with the catch curves of the three most abundant species of nonherring fish larvae that hatched into Auke Bay during the herbivorous maximum (Fig. 8B) (Haldorson et al. 1989) and with the density and mean wet weight of invertebrate planktonic predators on copepods (Fig. 8C) (Coyle and Paul 1989). The hypothesis predicts that the proportion of herring larvae classified as starving would increase directly with density of competitors. The ascending limbs and domes of the two catch curves coincided with the period during which starvation was measured: DOY 136–162. The only herring cohort that hatched into this period, and that spent its entire first-feeding phase during this period, was cohort 2. However, this evidence offers only partial support for the competition hypothesis because the proportion of starving larvae decreased during the period in which predator density increased. Also, the only way that predators

could have reduced the prey available for herring larvae was by interfering with herring foraging. There is little evidence that these taxa exhibit interference competition.

A third possible factor was that larvae classified as starving by CF were unable to feed because they were incompetent due to malformations induced during incubation. Abnormal herring larvae may have been produced by exposure of eggs to drying, direct sunlight, and extremes of air temperature in the intertidal zone (Purcell et al. 1990). This hypothesis has the advantage of explaining why the proportion of starving larvae in cohorts 2 and 3 decreased with DOY — it represents the loss of abnormal larvae. We tested the hypothesis that mean height of water at low tide, mean daily solar irradiance, and mean air and surface water temperatures were greater during the incubation period of cohort 2 than during the incubation periods of the other three cohorts. The data do not support this hypothesis — there were no differences ($P > 0.05$) among cohorts in mean daily solar irradiance or mean low tide, and mean air and surface water temperatures were highest for cohort 4, not cohort 2 (Table 6).

Although environmental conditions during egg incubation were not significantly different among cohorts, apart from a seasonal increase in air and surface water temperatures, eggs of cohort 2 and 3 could still have been exposed to much harsher incubation conditions than those experienced by eggs of cohorts 1 and 4 simply because they were laid in the upper intertidal zone. Eggs of cohorts 1 and 4 were probably laid almost entirely in subtidal habitat because their eggs were never found, despite intensive searches of the study area.

Dispersal and Mortality

Dispersal of herring larvae out of Auke Bay was indicated by the elongated patches of larvae with their major axes in a north–south direction and the fact that conversion of larval density to larval number had a significant effect on the estimate of larval loss/mortality.

However, at least three lines of evidence suggest that herring larvae did not disperse as passive particles, but were retained within Auke Bay or within the contiguous nearshore zone of Lynn Canal. First, there was no significant advection of larvae in a north–south direction; the only significant advection was westward along the northern shore of the Bay. Second, average westward advection of herring larvae was only 0.02 $cm \cdot s^{-1}$; at that velocity, a centroid would have moved only 1.2 km along the northern shore over the 60-d duration of the larval stage. It is difficult to reconcile such low advection with an exchange rate for Auke Bay that exeeds 300% over spring and summer and with episodes of water velocity that exceeded 5 $cm \cdot s^{-1}$ for several days at a time, unless some form of retention is invoked. Third, spatial variance of larval density in both the x- and y-axes was independent of age and date, so that diffusion, $s_t^2/4t$ (Okubo 1980), and shear, $((3s_{yt}^2/s_{xt}^2)^{0.5})/t$ (Munk et al. 1986), of herring larvae decreased inversely with age.

We do not attempt to reconcile the evidence for dispersal and retention because our study area was too small, and our sampling grid was too coarse, to support definitive statements. Much more extensive studies of larval herring dispersal in the Auke Bay – Lynn Canal area and in southeast Alaska are required to fully describe larval herring dispersal or retention. We described dispersal for the sole purpose of converting larval densities to larval numbers so as to accurately measure mortality.

Averge mortality of Auke Bay herring larvae falls within the lower range of mortalities previously reported for Pacific herring larvae (Stevenson 1962; Alderdice and Hourston 1985; McGurk 1989) and for Atlantic herring larvae (Munk et al. 1986; Heath and MacLachlan 1987; Kiørboe et al. 1988; Fortier and Gagné 1990). Mortality data do not support the food-limitation hypotheses because it was not higher during the first-feeding stage than during the yolk sac stage or late-larval stage and because there were no differences in mortality between cohorts, even though there were differences in growth and condition between cohorts. These conclusions must be qualified by stating that the statistical power of the regression of ln(number) on age was quite low.

Food Limitation?

We conclude that growth, condition, and mortality of herring larvae in Auke Bay in 1988 were not food limited. Therefore, this study offers only partial support for Cushing's (1975, 1990) match–mismatch hypothesis. It shows that the spawning strategy of herring in southeast Alaska means that their offspring are unlikely to entirely miss the spring herbivorous copepod maximum. However, because herring larvae in Auke Bay are dilute (a result of "trace" spawning), it is reasonable to suspect that food limitation may operate within high-density populations of herring larvae near major spawning beaches.

This study shows that there is a continuum of response of herring larval dynamics to food and that this continuum may occur within a population, in this case the Auke Bay – Lynn Canal stock, as well as between populations and between years. The Auke Bay cohorts are examples of low-density populations whose dynamics are not constrained by food, but by other factors such as incubation conditions and predation. Studies of such low-density populations can reveal mechanisms of population control not obvious from reseach on high-density populations.

Acknowledgments

This study was funded by the Alaska Outer Continental Shelf Region of the Minerals Management Service, U.S. Department of the Interior,

TABLE 6. Mean (±1 SD) air and water temperatures, irradiance, and height of low tide in Auke Bay, Alaska, during the incubation of four cohorts of herring eggs.

Cohort	Air temperature (°C)	Surface water temperature (°C)	Total daily irradiance (10^{21} quanta·cm^{-2}·d^{-1})	Low tide (m)[a]
1	7.2 (1.2)	7.3 (0.9)	3.42 (1.70)	0.5 (0.4)
2	10.0 (2.4)	10.1 (1.5)	3.31 (1.81)	0.4 (0.2)
3	11.9 (2.4)	11.1 (1.2)	3.83 (1.91)	0.5 (0.5)
4	12.4 (1.6)	12.0 (1.1)	3.43 (1.29)	0.9 (0.5)

[a]Above mean lower low water, only between the hours of 10:00 and 18:30.

Anchorage, Alaska, under contract No. 14-35-0001-30600 through an interagency agreement with the National Oceanic and Atmospheric Administration, U.S. Department of Commerce. K. O. Coyle, A. J. Paul, D. A. Ziemann, and L. J. Haldorson were involved through APPRISE (Association of Primary Production and Recruitment in Subarctic Ecosystems), a joint research program between the University of Alaska (Juneau Center for Fisheries and Ocean Sciences and the Institute for Marine Science, Fairbanks) and The Oceanic Institute of Honolulu, Hawaii, that was based in Auke Bay. It was supported by contract No. NA-85-ABH-00022 from the National Oceanic and Atmospheric Administration, U.S. Department of Commerce. We gratefully acknowledge the assistance of L. Jarvela, G. Stockdale, B. Wing, A. Wertheimer, C. Low, K. Bartness, E. Wilson, M. Stekoll, J. McConnaughey, and D. Tesch. The paper benefited from critical reviews by T. Kiørbe, J. D. Neilson, and several anonymous referees.

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Comparing Dynamic Versions of the Schaefer and Fox Production Models and Their Application to Lobster Fisheries

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Dynamic approaches (integrated and finite difference) to the Schaefer and Fox production models are applied to four commercial lobster fisheries. The integrated versions provided better predictions than their finite difference counterparts. Only the integrated version of the Fox model provides realistic (positive) biological parameter estimates for all four fisheries, and bootstrapping reveals those estimates to be generally stable. Additionally, this model performs well when applied to data where certain assumptions of surplus production modeling are fulfilled. The results suggest further investigation of the integration procedure.

Des méthodes dynamiques (méthode intégrée et méthode des différences finies) sont appliquées aux modèles de production de Schaefer et de Fox dans le cas de quatre pêches commerciales au homard. Les versions utilisant la méthode intégrée permettent d'obtenir de meilleures prévisions par rapport aux versions fondées sur la méthode des différences finies. Seule la version intégrée du modèle de Fox fournit des estimations réalistes (valables) des paramètres biologiques pour les quatre pêches, et l'application de la technique du *bootstrapping* démontre que ces estimations sont généralement stables. En outre, on obtient de bons résultats lorsque l'on applique ce modèle aux données qui confirment certaines hypothèses relativement aux modèles de production excédentaire. Ces résultats incitent donc à pousser les recherches du côté de la méthode intégrée.

Received November 26, 1990
Accepted July 29, 1992
(JA822)

Reçu le 26 novembre 1990
Accepté le 29 juillet 1992

One of the simplest approaches to modeling a fishery is applying a surplus production model to a time series of catch and effort data. Schaefer (1957) and Fox (1970) helped pioneer this approach and used the concept of equilibrium to model a fishery over the long run. They first applied a dynamic model to estimate various biological parameters of a fishery. The parameters were then used in an equilibrium model to estimate fishery management parameters (e.g. maximum sustainable yield). The dynamic models therefore had a dual purpose: to account for the nonequilibrium portions of a fishery and to accurately estimate the biological parameters so the resulting management parameters could be trusted. Many authors (Walter 1973; Schnute 1977; Uhler 1980; Lleonart and Salat 1989) have since developed their own dynamic versions of the Schaefer (1957) and Fox (1970) models to better assess unstabilized fisheries. The two dynamic approaches examined in this paper are the original (finite difference) versions and the integrated versions (Schnute 1977; Clarke et al. 1992).

The Schaefer (1957) and Fox (1970) models have the continuous forms

$$(1) \quad dX/dt = rX - rX^2/K - C$$

and

$$(2) \quad dX/dt = rX \ln(K/X) - C,$$

respectively, where X represents the population, dX/dt the growth rate of the population, C the catch rate, r the intrinsic growth rate, and K the maximum stock level or virgin biomass. Equation (1) assumes that the growth rate to biomass relationship is logistic (parabolic), while Equation (2) assumes a Gompertz distribution (Richards 1959).

Fishing effort is taken into consideration by substituting $C = qEX$, with q defined as the catchability coefficient and E as the rate of fishing effort (e.g. the effort expended in 1 yr). Equations (1) and (2) are then converted to

$$(3) \quad (1/U)dU/dt = r - r/(qK)U - qE$$

and

$$(4) \quad (1/U)dU/dt = r \ln(qK) - r \ln(U) - qE,$$

respectively, where $U = C/E$ is the instantaneous catch per unit effort (CPUE). Schaefer (1957) and Fox (1970) estimated parameters r, q, and K by converting Equations (3)and (4) into their finite difference forms:

Schaefer:

$$(5) \quad \Delta U_n/U_n = r - r/(qK)U_n - qE_n$$

Fox:

$$(6) \quad \Delta U_n/U_n = r \ln(qK) - r \ln(U_n) - qE_n$$

where U_n is the average CPUE and E_n is the total effort expended for year n and $\Delta U_n = (U_{n+1} - U_{n-1})/2$. Both Equations (5) and (6) are considered dynamic given that U_{n+1} can be expressed in terms of past variables U_n, E_n, and U_{n-1}. Originally, Equations (5) and (6) were used only to estimate parameters r, q, and K. However, we test the applicability of the dynamic models for predictions and parameter estimation in nonequilibrium conditions. Hereafter, Equations (5) and (6)

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will be called the FD (finite difference) - Schaefer and FD-Fox models, respectively.

Upon scrutiny, the FD-Schaefer and FD-Fox models possess some questionable properties. One problem is the approximation $dU/dt = \Delta U_n$, which assumes that CPUE is linearly distributed over the course of 2 yr. Another problem is that the CPUE of year $n + 1$, U_{n+1}, can be predicted without the anticipated effort being specified for that year. Such a condition is questionable, given the definition of CPUE: $U = C/E$.

Rather than using a finite difference approximation, Schnute (1977) integrated Equation (3) and approximated the average annual CPUE as the geometric mean of the instantaneous CPUEs at the beginning and end of the year. Schnute's dynamic version of the Schaefer model, henceforth referred to as the Schnute model, is

$$(7)\quad \ln(U_{n+1}/U_n) = r - (r/(qK))(U_n + U_{n+1})/2 - q(E_n + E_{n+1})/2.$$

As noted by Uhler (1980), the Schnute model, unlike the FD-Schaefer and FD-Fox models, has the desirable property that U_{n+1} tends to zero as E_{n+1} becomes large. Another advantage of this model is that the predicted CPUE, U_{n+1}, can be estimated for a range of anticipated effort, E_{n+1}, to allow fishery managers to approximate the effort level needed for a targeted yield. In Uhler (1980), the Schnute model was shown to have less bias than an FD-Schaefer model (with a 1-yr time lag) when applied to a computer-simulated fishery.

An integrated version of the Fox model has been applied to the lobster fishery in the Northwestern Hawaiian Islands (NWHI) (Clarke et al. 1992). This model uses the same assumptions as the Schnute model and incorporates a Taylor series approximation (Appendix A):

$$(8)\quad \ln(U_{n+1}) = [2r\ln(qK) + (2 - r)\ln(U_n) - q(E_n + E_{n+1})]/(2 + r).$$

Unlike the other models presented, this model is a simple, lagged logarithmic equation of CPUE. This gives the model an advantage over the Schnute and finite difference models when regression analysis is performed. This model has been applied to a limited time series of catch and effort data (8 yr of the NWHI lobster fishery; Clarke et al. 1992), but its applicability to fisheries with longer time series is unknown. Hereafter, this model will be called the I (integrated) - Fox model.

Our paper examines the assumption that the integrated models will yield better predictions and biological parameter estimates than the finite difference models. The models are applied to four commercial lobster fisheries with substantial time series of catch and effort data, and each model's ability to predict annual CPUE is investigated. Biological parameter estimates for each fishery also are presented. However, since little is known of these parameters, only general conclusions are made about them. Each model's ability for parameter estimation is tested using data sets in which the biological parameter q is known.

Methods

A frequently used approach to estimate a model's predictive power of catch is applying an ordinary least squares (OLS) regression on all years of data for a fishery. The points on the resulting regression curve are then interpreted as catch (or CPUE) predictions for the original data. Wittink (1988) pointed out that this approach depends heavily on the R-squared (R^2) value and does not test for predictive power, since the regression curve is created using the original data (i.e. the curve has prior knowledge of what it is supposed to predict).

The approach used in this paper is to make annual predictions only using data prior to that year. Mathematically, OLS is performed on the first $m - 1$ years of data, and a prediction is made for year m using the results of that regression. A regression is then performed on the first m years for the prediction of year $m + 1$, and this procedure is continued until a prediction is made for the last year of available data. The predicted and actual values are then compared to determine predictive power. This approach better approximates what fishery managers actually face because having data for future years is unrealistic.

Each model's estimates of the biological parameters r, q, and K, using all years of data for a fishery, are also examined. From definitions presented in Schnute (1977), each biological parameter should be positive. The parameter estimates presented in our paper are unconstrained estimates. Other papers (e.g. Schaefer 1957) take absolute values to ensure the analyses have realistic (positive) results. We use the unconstrained approach to look at the mathematical relations of the models rather than to thoroughly analyze a specific fishery.

The four dynamic models are applied to catch and effort data from four lobster fisheries: Western Australian rock lobster, *Panulirus cygnus* (years 1944–64 from table 1 in Morgan 1979a; years 1965–78 from tables 6 and 8 in Morgan et al. 1982); American lobster, *Homarus americanus* (years 1950–79 from table 1 in Townsend 1986); Tasmanian rock lobster, *Jasus novaehollandiae* (years 1947–84 from table 1 in Campbell and Hall 1988); and New Zealand lobster, *Jasus edwardsii* (years 1945–90 from the totals in table 1 in Breen 1991). The Western Australian data take into account seasonal variability and area distribution (Morgan 1979b). The New England data are only inshore data, and a modified Schaefer model was applied to them in Townsend (1986). An FD-Fox model and an FD-Schaefer model with 1-yr time lags have been applied to the Tasmanian data (Campbell and Hall 1988). Finally, the New Zealand data are composed of the total of commercial and estimated unreported, amateur, and illegal catch data. To our knowledge, each data set has not been proven to satisfy all assumptions of surplus production modeling. However, the applicability of these models to commercial data can be gauged by each model's ability to forecast catch (or CPUE).

A prediction for the CPUE of year $m + 1$ is made in the following manner. An OLS regression is applied to the models by using catch and effort data for the first m years of a fishery. The resulting regression on data for years $n = 1$ to $n = m$ estimates the parameters r, q, and K:

FD-Schaefer:

$$(9)\quad \Delta U_{n-1}/U_{n-1} = c1 + c2U_{n-1} + c3E_{n-1}$$

with $r = c1$, $q = -c3$, and $K = -r/(qc2)$;

FD-Fox:

$$(10)\quad \Delta U_{n-1}/U_{n-1} = c1 + c2\ln(U_{n-1}) + c3E_{n-1}$$

with $r = -c2$, $q = -c3$, and $K = e^{c1/r}/q$;

Schnute:

$$(11)\quad \ln(U_n/U_{n-1}) = c1 + c2(U_{n-1} + U_n)/2 + c3(E_{n-1} + E_n)/2$$

with $r = c1$, $q = -c3$, and $K = -r/(qc2)$; and

I-Fox:

$$(12)\quad \ln(U_n) = c1 + c2\ln(U_{n-1}) + c3(E_{n-1} + E_n)$$

with $r = 2(1 - c2)/(1 + c2)$, $q = -c3(2 + r)$, and $K = e^{c1(2+r)/(2r)}/q$. The FD-Schaefer and FD-Fox models predict the following year's CPUE with

FD-Schaefer:

$$(13)\quad U_{m+1} = U_{m-1} + 2U_m[r - r/(qK)\,U_m - qE_m]$$

and

FD-Fox:

$$(14)\quad U_{m+1} = U_{m-1} + 2U_m[r\ln(qK) - r\ln(U_m) - qE_m].$$

For the I-Fox model, after the anticipated effort E_{m+1} is specified, the predicted CPUE is

$$(15)\quad U_{m+1} = e^{\frac{(2r\ln(qK) + (2 - r)\ln(U_m) - q(E_m + E_{m+1}))}{(2 + r)}}.$$

For the Schnute model, U_{m+1} was estimated from Equation (31) in Schnute (1977). Confidence intervals for the Schnute model's prediction are in Schnute (1977); those for the I-Fox are in Appendix B.

The predictive ability of the models is tested by using Theil's U-statistic (Wittink 1988):

$$(16)\quad U_T = \sqrt{\frac{\sum_{n=k}^{N}(U_n - U_{p,n})^2}{\sum_{n=k}^{N}(U_n - U_{n-1})^2}}$$

where $U_{p,n}$ is the predicted CPUE for year n, and k and N are equal to the first and last year, respectively, that a CPUE prediction was made. The numerator of U_T is the sum of the squared differences of the actual and predicted CPUEs, and the denominator is the sum of the squared differences between the actual CPUEs of adjacent years. Thus, this statistic adjusts a model's predictive error by considering the year-to-year variation of the actual CPUE. If $U_T > 1$, the model is not considered useful for forecasting purposes.

For the overall estimation of biological parameters (r, q, and K), the following procedure is used. First, regressions of the four models are performed on all years of data. The presence of autocorrelation is then investigated. Since the I-Fox model has a lagged dependent variable, the Durbin–Watson statistic is inappropriate to detect autocorrelation, and the test statistic used is the Durbin h-statistic (Pindyck and Rubinfeld 1981). When the Durbin h-statistic is undefined, the Durbin t-statistic test is applied (King 1987). If the FD-Schaefer, FD-Fox, or Schnute model passes either the Durbin h-test or the regular Durbin–Watson test, then the results of that model's regression are used. When evidence of autocorrelation is detected, the Cochrane–Orcutt procedure for autocorrelation correction (Wittink 1988) is applied until the model's test statistic is not significant in showing autocorrelation. The model loses a degree of freedom for each Cochrane–Orcutt iteration performed.

TABLE 1. Average percent prediction errors from the I-Fox, Schnute, FD-Fox, and FD-Schaefer models for four lobster fisheries.

Location	I-Fox	Schnute	FD-Fox	FD-Schaefer
New England	11.58	10.35	18.56	18.56
New Zealand	18.86	16.58	20.92	20.62
Tasmania	12.20	12.26	21.54	21.01
Western Australia	12.49	12.75	25.69	24.26

TABLE 2. Theil's U-statistic test applied to the I-Fox, Schnute, FD-Fox, and FD-Schaefer models for four lobster fisheries.

Location	I-Fox	Schnute	FD-Fox	FD-Schaefer
New England	0.780[a]	0.750[a]	1.44	1.43
New Zealand	1.86	3.20	2.46	2.43
Tasmania	0.827[a]	0.910[a]	1.60	1.55
Western Australia	1.01	1.21	2.70	2.45

[a]Model is considered an adequate predictor.

Results and Discussion

The lack of degrees of freedom for regression analysis with the FD-Fox and FD-Schaefer models, which have a 2-yr time lag, prevented predictions from being made for the first 6 yr of each fishery. Therefore, CPUE predictions for years 1956–79, 1951–90, 1953–84, and 1950–78 were obtained for the New England, New Zealand, Tasmanian, and Western Australian lobster fisheries, respectively. A model's CPUE prediction for the seventh year used the regression results from the first 6 yr of data, the prediction for the eighth year used those from the first 7 yr of data, and so on.

The percent prediction error

$$(17)\quad 100\,|U_n - U_{p,n}|/U_n$$

was calculated for each model's prediction of CPUE, with $U_{p,n}$ being the predicted CPUE for year n. The means of the percent prediction errors from each model (Table 1) show that the I-Fox model is the most accurate predictor for two of the four fisheries, and the Schnute model is the more accurate predictor for the other two. In all four fisheries, the I-Fox and Schnute models have lower average percent prediction errors than the finite difference models. According to Theil's U-statistic test (Table 2), the I-Fox and Schnute models are adequate predictors for the Tasmanian and New England fisheries, while the FD-Fox and FD-Schaefer models failed the test for all four fisheries.

From the regressions on all data of a fishery, only the I-Fox model provides positive biological parameters for all four fisheries (Tables 3–6). The I-Fox model also had high R^2 values (>0.8), but this goodness of fit was expected. The low R^2 values from the other three models reveal problems in explaining the variation in their more complex dependent variables ($\Delta U_n/U_n$ for the FD-Fox and FD-Schaefer models and $\ln(U_{n+1}/U_n)$ for the Schnute model) over substantially long time series. This was probably a major reason for their unrealistic (negative) estimates of the biological parameters. Another possible explanation is that the data sets examined do not satisfy the assumptions of surplus production modeling. The higher R^2 values of the I-Fox model compared with those of the Schnute model reveal that a better regression fit does not necessarily imply better predictive power (Ferber 1956).

Bootstrapping (Efron and Tibshirani 1986) was applied to the I-Fox model to test the stability of the biological parameters. Sampling with replacement was applied to the residuals of each

TABLE 3. Final regression coefficients and parameter estimates for the New England inshore lobster fishery (NA = not applicable). *$P < 0.05$.

	I-Fox	Schnute	FD-Fox[a]	FD-Schaefer[a]
$c1$	−1.62	−0.444	0.399	−0.308
t-ratio for $c1$	−2.57*	−1.40	0.742	−1.11
$c2$	0.416	7.94	0.176	4.68
t-ratio	1.79	1.28	0.911	0.830
$c3$	-2.41×10^{-4}	1.61×10^{-4}	1.75×10^{-4}	1.15×10^{-4}
t-ratio for $c3$	−2.42*	1.26	1.07	1.04
df	26	26	24	24
R^2	0.943	0.0606	0.0493	0.0439
R^2 adj	0.939	−0.0117	−0.0299	−0.0358
Durbin h	Undefined	Undefined	Undefined	Undefined
Durbin t	1.32*	NA	NA	NA
Durbin–Watson	1.19	1.95*	1.69*	1.70*
r (yr^{-1})	0.826	−0.444	−0.176	−0.308
q (1000 $traps^{-1}$)	6.80×10^{-4}	-1.61×10^{-4}	-1.75×10^{-4}	-1.15×10^{-4}
K (millions of pounds)	92.6	−347	−591	−571

[a]One iteration of the Cochrane–Orcutt procedure was applied.

TABLE 4. Final regression coefficients and parameter estimates for the New Zealand inshore lobster fishery (NA = not applicable). *$P < 0.05$.

	I-Fox[a]	Schnute[b]	FD-Fox	FD-Schaefer
$c1$	0.369	−0.0773	−0.0446	−0.0523
t-ratio for $c1$	2.18*	−0.539	−0.366	−0.464
$c2$	0.718	0.0158	0.0165	0.00875
t-ratio for $c2$	6.06*	0.391	0.192	0.281
$c3$	-4.24×10^{-5}	2.01×10^{-6}	-1.30×10^{-6}	-1.58×10^{-6}
t-ratio for $c3$	−2.41*	0.0902	−0.0502	−0.0870
df	40	41	41	41
R^2	0.934	0.0108	0.00974	0.0108
R^2 adj	0.930	−0.0374	−0.0386	−0.0375
Durbin h	−1.05*	−0.163*	1.31*	1.09*
Durbin–Watson	NA	2.05*	1.68*	1.68*
r (yr^{-1})	0.329	−0.0773	−0.0165	−0.0523
q (1000 $pot\text{-}lifts^{-1}$)	9.88×10^{-5}	-2.01×10^{-6}	1.30×10^{-6}	1.58×10^{-6}
K (tonnes)	37 469	−2 440 845	11 527 340	3 788 069

[a]Two iterations of the Cochrane–Orcutt procedure were applied.
[b]One iteration of the Cochrane–Orcutt procedure was applied.

TABLE 5. Final regression coefficients and parameter estimates for the Tasmanian lobster fishery (NA = not applicable). *$P < 0.05$.

	I-Fox	Schnute	FD-Fox	FD-Schaefer
$c1$	6.40	0.494	1.34	0.272
t-ratio for $c1$	5.41*	1.36	0.978	1.03
$c2$	0.256	-1.14×10^{-4}	−0.162	-6.64×10^{-5}
t-ratio for $c2$	1.89	−1.66	−1.02	−1.29
$c3$	−0.416	−0.311	−0.135	−0.174
t-ratio for $c3$	−4.30*	−1.11	−0.660	−0.889
df	34	34	33	33
R^2	0.833	0.0953	0.0452	0.0624
R^2 adj	0.823	0.0421	−0.0126	0.00556
Durbin h	0.976*	−1.35*	5.46	1.52*
Durbin–Watson	NA	2.44	1.44	1.49
r (yr^{-1})	1.19	0.494	0.162	0.272
q (million $pot\text{-}days^{-1}$)	1.33	0.311	0.135	0.174
K (tonnes)	4079	13 997	29 056	23 467

of the four I-Fox data sets (a Cochrane–Orcutt-transformed data set was used for the New Zealand fishery), and a regression was performed on each new data set. This procedure was replicated 1000 times with each regression yielding new estimates of r, q, and K. The mean, standard deviation, and coefficient of variation of the r, q, and K bootstrap estimates for each fishery are shown in Table 7. The coefficients of variation suggest that the parameters derived from the I-Fox model are relatively stable for all parameters except K (high variability was shown in the New England and New Zealand lobster fisheries).

The integrated models predict CPUE reasonably (<20% error) for all four lobster fisheries but fail to pass Theil's U-

TABLE 6. Final regression coefficients and parameter estimates for the Western Australian lobster fishery (NA = not applicable). *$P < 0.05$.

	I-Fox	Schnute	FD-Fox[a]	FD-Schaefer[a]
$c1$	0.617	0.106	−0.284	−0.411
t-ratio for $c1$	3.73*	0.304	−1.42	−1.58
$c2$	0.429	−0.0369	0.209	0.127
t-ratio for $c2$	2.88*	−0.297	1.22	1.41
$c3$	−0.0354	−0.0113	0.032	0.0332
t-ratio for $c3$	−3.67*	−0.410	1.33	1.51
df	31	31	29	29
R^2	0.882	0.00725	0.0572	0.0735
R^2 adj	0.875	−0.0568	−0.00781	0.00955
Durbin h	1.58*	0.144*	2.58	0.608*
Durbin–Watson	NA	1.97*	1.78*	1.81*
r (yr^{-1})	0.799	0.106	−0.209	−0.411
q (million pot-lifts^{-1})	0.0991	0.0113	−0.0317	−0.0332
K (millions of kilograms)	29.7	253	−123.4	−98.0

[a]One iteration of the Cochrane–Orcutt procedure was applied.

TABLE 7. Bootstrap estimates of the means, standard deviations (SD), and coefficients of variation (CV) of parameter r, q, and K when applying the I-Fox model to four lobster fisheries.

Parameter	Mean	SD	CV
	New England		
r	0.912	0.509	0.56
q	0.000755	0.000430	0.57
K	120.2	399.8	3.33
	New Zealand		
r	0.340	0.170	0.50
q	0.000102	0.0000513	0.50
K	44 061	96 482	2.19
	Tasmania		
r	1.23	0.368	0.30
q	1.38	0.469	0.34
K	4355	1573	0.36
	Western Australia		
r	0.838	0.316	0.38
q	0.103	0.0400	0.39
K	33.7	18.6	0.55

statistic for the New Zealand and Western Australian fisheries. Also, despite providing positive, unconstrained estimates of the biological parameters, instability with the I-Fox model is shown in parameter K. These findings could indicate a theoretical problem with all of the models investigated: the assumption that $C = qEX$. Since the estimates of biomass are not known, verification of this assumption is difficult. However, data sets designed to fit this assumption exist. The applicability of the I-Fox model toward these data sets is explored.

Application to the Silliman and Gutsell (1958) Data Sets

Guppy populations (*Lebistes reticulatus*) were introduced into four similarly designed tanks, and two (called populations A and B) were "fished" with a certain percent removal during each 3-wk interval (Silliman and Gutsell 1958). Fishing effort data were computed so that $U = qX$ with $q = 0.1$. Since two tanks were unfished, the maximum stock level each tank achieved could be thought of as estimates of the virgin biomass K. The maximum weight attained in tanks C and D equaled 37.1 and 36.0 g, respectively. This suggests that reasonable estimates of K for populations A and B are probably in the range of 30–40 g. Pella and Tomlinson (1969) estimated $q = 0.071$ and $K = 49.3$ for population A and $q = 0.078$ and $K = 39.5$ for population B.

Tables 8 and 9 present the catch and effort data along with estimates of q, K, and predicted CPUE U_p from the I-Fox model for each 3-wk interval. The catch data are from Silliman and Gutsell 1958) whereas the effort data are adjusted by scaling the percent weight removals as suggested in Pella and Tomlinson (1969). Each estimate of q, K, and U_p is obtained from the I-Fox regression on all data prior to that 3-wk interval. Because of a lack of degrees of freedom, the I-Fox regression estimates could not be obtained for the first four observations of each table.

The tables present some revealing points of the I-Fox model. For example, the initial estimates of q and K are unrealistic (negative). This could be due either to the lack of data or to the effort levels for the first 13 observations being in a narrow range. Also, despite obtaining negative values of q and K, the I-Fox model still predicted CPUE accurately. This implies that a model that estimates biological parameters poorly could still prove useful when predicting future catch (e.g. the Schnute model applied to the New England lobster fishery). Finally, the I-Fox model's estimates become unrealistic for the last observations, suggesting that the model encounters problems when applied to fisheries nearing extinction.

The main point of the tables is that the I-Fox model accurately assesses "fisheries" A and B for a large portion of their life spans. In both populations, estimates of q and K become fairly stable by week 100, with the estimates of population B hovering around the "true" values of q (0.1) and K (between 30 and 40). Considering the I-Fox model is an adequate predictor of CPUE (Theil's U-statistic was 0.751 for population A and 0.781 for population B), the model seems to be a valuable management tool for fisheries that satisfy the assumption of $C = qEX$.

The ability of the other three models to estimate parameter q has also been analyzed. As was done in Tables 8 and 9 with the I-Fox model, values of q were obtained from each model (Fig. 1). The deviation of each q estimate from 0.100,

TABLE 8. Predicted CPUE U_p, prediction error, and parameters K and q from the I-Fox model for each 3-wk interval in the population A data set of Silliman and Gutsell (1958).

Week	Catch	Effort	CPUE	U_p	% error	K	q
40	6.0	2.58	2.33				
43	4.9	2.49	1.97				
46	3.7	2.06	1.80				
49	4.4	2.50	1.76				
52	4.1	2.43	1.69	1.82	7.69	−2.9	−0.326
55	3.5	2.15	1.63	1.68	3.07	30.1	0.072
58	3.5	2.32	1.51	1.63	7.95	35.6	0.058
61	3.8	2.59	1.47	1.51	2.72	−98.5	−0.014
64	3.3	2.36	1.40	1.45	3.57	41.0	0.049
67	3.3	2.23	1.48	1.40	5.41	31.5	0.099
70	2.8	1.90	1.47	1.52	3.40	26.5	0.131
73	3.0	2.00	1.50	1.50	0.00	31.0	0.079
76	3.9	2.62	1.49	1.49	0.00	31.5	0.077
79	1.4	1.05	1.33	1.52	14.29	31.5	0.076
82	2.1	1.41	1.49	1.29	13.42	−15.5	−0.064
85	1.9	1.23	1.54	1.53	0.65	39.4	0.050
88	2.0	1.33	1.50	1.58	5.33	37.7	0.054
91	1.7	1.06	1.60	1.53	4.38	51.1	0.035
94	1.5	0.90	1.67	1.64	1.80	40.0	0.049
97	1.9	1.06	1.79	1.69	5.59	37.6	0.054
100	1.7	0.97	1.75	1.81	3.43	34.2	0.067
103	1.2	0.62	1.94	1.79	7.73	35.0	0.062
106	2.0	0.94	2.13	1.96	7.98	33.2	0.075
109	2.0	0.87	2.30	2.14	6.96	35.5	0.084
112	2.6	1.12	2.32	2.31	0.43	42.3	0.089
115	2.9	1.23	2.36	2.29	2.97	43.2	0.089
118	3.0	1.31	2.29	2.32	1.31	47.0	0.089
121	11.2	4.77	2.35	1.95	17.02	45.3	0.089
124	7.0	4.58	1.53	1.91	24.84	88.7	0.063
127	5.2	4.37	1.19	1.21	1.68	51.6	0.097
130	5.7	5.09	1.12	0.95	15.18	52.8	0.098
133	5.6	5.05	1.11	0.93	16.22	40.3	0.088
136	4.2	4.38	0.96	1.00	4.17	36.7	0.079
139	4.3	4.78	0.90	0.89	1.11	37.2	0.080
142	3.5	4.32	0.81	0.85	4.94	37.0	0.080
145	3.9	5.42	0.72	0.76	5.56	38.2	0.080
148	3.5	5.00	0.70	0.67	4.29	39.7	0.080
151	3.4	6.54	0.52	0.63	21.15	38.3	0.080
154	2.1	7.00	0.30	0.44	46.67	42.8	0.082
157	2.4	7.27	0.33	0.24	27.27	71.3	0.084
160	1.2	6.32	0.19	0.30	57.89	39.3	0.092
163	1.2	6.67	0.18	0.17	5.56	71.8	0.083
166	0.9	6.92	0.13	0.16	23.08	60.5	0.087
169	1.0	7.14	0.14	0.11	21.43	91.9	0.080
172	0.2	3.33	0.06	0.15	150.00	59.7	0.088

$|q - 0.1|$, was then computed for each 3-wk interval after week 82 (the last week that the models estimated a negative value of q). The average deviations for each model reveal that the integrated models are superior to the finite difference models in estimating q. For population A, the average deviations for the I-Fox and Schnute models are 0.024 and 0.029, respectively. The FD-Fox and FD-Schaefer models estimate q more poorly; the average deviation is 0.043 for each model. For population B, the I-Fox and Schnute models estimate q more accurately, with average deviations of 0.012 and 0.013, respectively. Although the FD-Schaefer and FD-Fox models are also more accurate in population B than in population A (0.030 and 0.033 average deviations, respectively), their q estimates are still not as accurate as the integrated models.

Conclusion

Ideally, fishery managers require a model that accurately predicts catch (or CPUE) while also estimating reasonable biological parameters. None of the models performed exceptionally well when applied to the four commercial lobster data sets, although a case could be made for the applicability of the I-Fox model to the Tasmanian lobster fishery. The model passed Theil's U-statistic when predicting CPUE and had positive (and stable) biological parameters. Yet specific instances of inaccurate CPUE predictions by the I-Fox model were still found. This supports the consensus that surplus production models are too simple to accurately reflect fish and fishery interactions. Temperature, spawning stock information, and operational

TABLE 9. Predicted CPUE U_p, prediction error, and parameters K and q from the I-Fox model for each 3-wk interval in the population B data set of Silliman and Gutsell (1958).

Week	Catch	Effort	CPUE	U_p	% error	K	q
40	8.0	2.60	3.08				
43	5.0	2.13	2.35				
46	5.6	2.55	2.20				
49	5.6	2.86	1.96				
52	4.5	2.57	1.75	1.89	8.00	12.0	0.417
55	4.0	2.50	1.60	1.84	15.00	14.4	0.453
58	3.1	2.18	1.42	1.61	13.38	24.8	0.142
61	3.5	2.57	1.36	1.35	0.74	−12.0	−0.053
64	3.5	2.63	1.33	1.32	0.75	−15.7	−0.046
67	3.7	2.50	1.48	1.30	12.16	−14.4	−0.049
70	3.6	2.45	1.47	1.44	2.04	−9.6	−0.078
73	3.4	2.30	1.48	1.43	3.38	−10.7	−0.074
76	3.8	2.70	1.41	1.46	3.55	−18.6	−0.052
79	1.5	1.13	1.33	1.36	2.26	−18.5	−0.051
82	1.9	1.22	1.56	1.22	21.79	−8.7	−0.084
85	1.9	1.17	1.62	1.65	1.85	30.4	0.083
88	2.1	1.30	1.62	1.68	3.70	31.2	0.078
91	2.3	1.42	1.62	1.64	1.23	33.3	0.067
94	2.2	1.26	1.75	1.64	6.29	34.0	0.064
97	1.9	1.02	1.86	1.77	4.84	31.8	0.074
100	2.3	1.10	2.09	1.89	9.57	30.7	0.083
103	2.4	1.09	2.20	2.09	5.00	30.3	0.098
106	2.8	1.24	2.26	2.18	3.54	31.1	0.104
109	2.7	1.14	2.37	2.23	5.91	31.9	0.107
112	3.3	1.39	2.37	2.32	2.11	33.7	0.111
115	1.9	0.89	2.13	2.36	10.80	34.5	0.112
118	2.2	1.02	2.16	2.19	1.39	31.9	0.107
121	10.2	4.66	2.19	1.85	15.53	31.8	0.106
124	7.0	4.70	1.49	1.67	12.08	38.2	0.086
127	6.8	5.62	1.21	1.13	6.61	33.8	0.105
130	5.3	5.64	0.94	0.95	1.06	34.1	0.097
133	4.3	4.67	0.92	0.81	11.96	34.1	0.098
136	4.8	4.80	1.00	0.86	14.00	32.1	0.094
139	4.2	4.94	0.85	0.93	9.41	30.5	0.092
142	3.5	5.07	0.69	0.81	17.39	30.9	0.094
145	2.6	4.81	0.54	0.68	25.93	32.5	0.095
148	2.8	4.52	0.62	0.54	12.90	38.0	0.092
151	3.7	6.98	0.53	0.57	7.55	33.7	0.096
154	2.9	6.44	0.45	0.46	2.22	34.6	0.097
157	1.2	6.00	0.20	0.42	110.00	34.8	0.097
160	1.7	7.73	0.22	0.17	22.73	64.6	0.092
163	1.1	8.46	0.13	0.18	38.46	42.7	0.101
166	0.3	6.00	0.05	0.12	140.00	54.9	0.101
169	0.2	10.00	0.02	0.03	50.0	4.6×10^{44}	0.071

changes should be incorporated to better approximate the dynamics of a fishery. Both integrated models, however, show adequate predictive power for the four lobster fisheries and estimate q accurately for the Silliman and Gutsell (1958) data sets. We believe that as mentioned in Silliman (1971), surplus production models can be valuable as first approximations and should be considered in time- or data-limiting conditions.

More sophisticated nonlinear approaches to the FD-Schaefer, FD-Fox, and Schnute models are found in Pella and Tomlinson (1969), Fox (1975), and Schnute (1977), respectively. These observation error fitting procedures (Hilborn and Walters 1992) use an iterative procedure with a least squares criterion to obtain estimates of r, q, and K which minimize predictive error. The parameters obtained from the linear models examined here are first approximations to that criterion. Using simulated populations, Hilborn (1979) showed that the nonlinear FD-Schaefer and Schnute models produced positive parameters, while their linear counterparts gave negative results; however, the nonlinear methods were not shown to assess those fisheries any better than the linear methods. A nonlinear approach applied to the I-Fox model could possibly produce better results, but this is beyond the scope of the current study. A more complete discussion on the inclusion of a model's observed error structure is found in Schnute (1989).

The present paper has compared two linear dynamic approaches with the Schaefer and Fox production models. The results suggest that the integration approach of Schnute (1977) should be explored more extensively. For example, the integration procedure could incorporate a longer time lag (Walter 1973) to account for the recruitment patterns of lobster. A 4-yr period was shown to work for the Western Australian lobster fishery in Phillips (1986). Additionally, the integration approach could be applied to a more general class of curves, such as the Bernoulli curves used in Pella and Tomlinson (1969), with the chosen curve satisfying some statistical criterion. Research in these areas could lead to a better under-

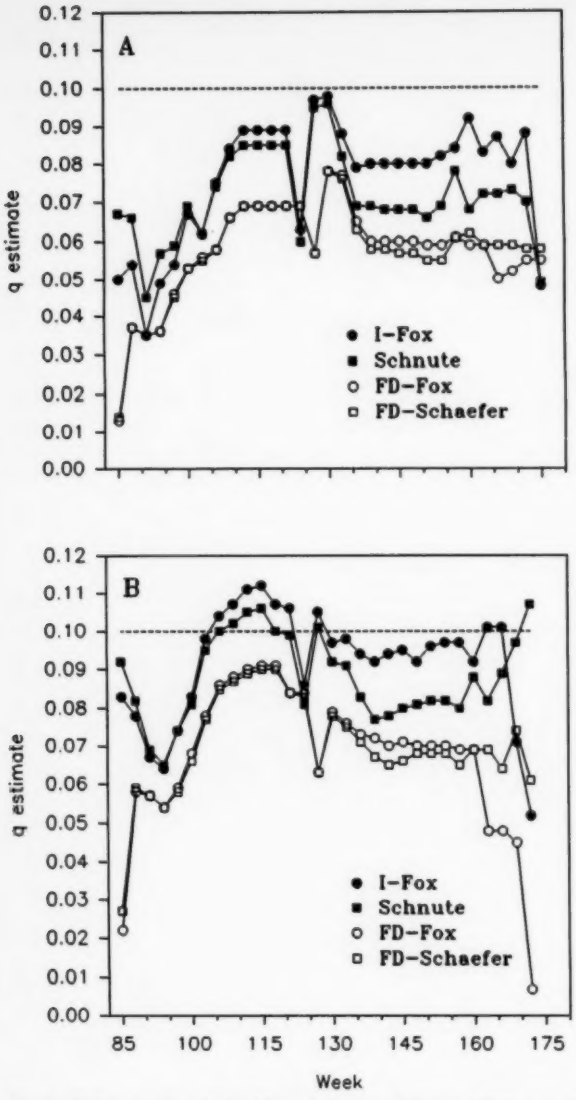

FIG. 1. Estimates of q obtained from the I-Fox, Schnute, FD-Fox, and FD-Schaefer models for the population (A and B) data sets of Silliman and Gutsell (1958)

standing of the relative usefulness of surplus production modeling in specific fishery applications.

Acknowledgements

We thank Samuel G. Pooley for his help in this project. Also, thanks go to Paul A. Breen, Jerome J. Pella, Robert A. Skillman, and Gilbert G. Walter for their constructive reviews. Finally, many thanks to Frank Cabacungan, Kathleen Clarke, Michael Seki, and Leslie Williams for their input in making this paper more presentable.

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Appendix A. Derivation of the I-Fox Model

Integrating Equation (4) from t = year n to t = year $n + 1$ yields

$$\text{(A1)}\quad \ln(U<n+1>/U<n>) = r\ln(qK) - r\int_{t=n}^{n+1} \ln(U)\,dt - qE_n$$

where $U<n>$ is the instantaneous CPUE at the start of year n and E_n is the total effort for year n.

The first-degree Taylor polynomial for $\ln(U)$ centered at U_n, the average CPUE for year n, is

$$\ln(U) \approx \ln(U_n) + (1/U_n)(U - U_n)$$
$$= \ln(U_n) - 1 + (U/U_n).$$

This approximation assumes that U will not fluctuate far from U_n over the course of year n. Thus, high variability of instantaneous CPUE due to seasonal effects may render this assumption (and the I-Fox model) invalid for a specific fishery.

Integration of the Taylor approximation yields

$$\text{(A2)}\quad \int_n^{n+1} \ln(U)\,dt = \ln(U_n) - 1 + (1/U_n)\int_n^{n+1} U dt.$$

By definition, $U_n = \int_n^{n+1} U dt$, so Equation (A2) becomes

$$\int_n^{n+1} \ln(U)\,dt = \ln(U_n) - 1 + 1 = \ln(U_n).$$

Putting this result into Equation (A1) gives

$$\ln(U<n+1>/U<n>) = r\ln(qK) - r\ln(U_n) - qE_n.$$

Adding this equation to its corresponding $(n + 1)$th equation gives

$$\text{(A3)}\quad \ln((U<n+2>\,U<n+1>)/(U<n+1>\,U<n>)) = 2r\ln(qK) - r(\ln(U_{n+1}) + \ln(U_n)) - q(E_n + E_{n+1}).$$

Schnute's (1977) assumption is used to estimate instantaneous CPUE:

$$U_n = \sqrt{U<n+1>\,U<n>},$$

that is, the CPUE of a given year is the geometric mean of the CPUEs at the beginning and ending of that year. Using this CPUE estimate in Equation (A3) and solving algebraically for $\ln(U_{n+1})$ gives

$$\ln(U_{n+1}) = (2r/(2+r))\ln(qK) + ((2-r)/(2+r))\ln(U_n) - (q/(2+r))(E_n + E_{n+1}).$$

Appendix B. Confidence Intervals for the I-Fox Model's Prediction of Catch per Unit Effort

The standard error of forecast S_f for the I-Fox model's prediction of CPUE for year $m + 1$ is found by applying the procedure in Ezekiel and Fox (1959):

$$S_f = \sqrt{S_1^2\left(1 + \frac{1}{m-1} + c_{XX}(X_m - M_X)^2 + 2c_{XY}(X_m - M_X)(Y_m - M_Y) + c_{YY}(Y_m - M_Y)^2\right)}$$

where $X_n = \ln(U_n)$, $Y_n = E_n + E_{n+1}$, $Z_n = \ln(U_{n+1})$, $M_X = \Sigma X_n/(m-1)$, $M_Y = \Sigma Y_n/(m-1)$, $S_X = \Sigma(X_n - M_X)^2$, $S_Y = \Sigma(Y_n - M_Y)^2$, $S_{XY} = \Sigma(X_n - M_X)(Y_n - M_Y)$, $c_{XX} = S_Y/(S_X S_Y - S_{XY}^2)$, $c_{XY} = -S_{XY}/(S_X S_Y - S_{XY}^2)$, $c_{YY} = S_X/(S_X S_Y - S_{XY}^2)$, $S_1^2 = \Sigma(Z_n - c1 - c2X_n - c3Y_n)^2/(m-4)$, and m is the number of years of data used. All sums (Σ) are from $n = 1$ to $n = m - 1$, and $c1$, $c2$, and $c3$ are the regression coefficients of the I-Fox regression. Also, the number of degrees of freedom is $m - 4$ because of the 1-yr time lag.

Using the formula for S_f, a confidence interval for $\ln(U_{m+1})$ can be calculated:

Lower limit: $l = \ln(U_{m+1}) - S_f t_{p/2}$

and

Upper limit: $u = \ln(U_{m+1}) + S_f t_{p/2}$

where $t_{p/2}$ is the critical value of the Student's t-distribution statistic of $p/2$ probability.

Since the natural log function $\ln(U)$ is an increasing function and is well defined for $U > 0$, confidence limits for the prediction U_{m+1} can be estimated by taking the inverse (exponent) of the confidence limits for $\ln(U_{m+1})$:

Lower limit: e^l

and

Upper limit: e^u.

Sedimentary Phosphorus in the Bay of Quinte, Lake Ontario

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Oluyedun, O. A., S. O. Ajayi, G. W. vanLoon, and P. Sly. 1993. Sedimentary phosphorus in the Bay of Quinte, Lake Ontario. Can. J. Fish. Aquat. Sci. 50: 190–197.

Phosphorus distributions in sediments of the Bay of Quinte were measured. Principal fractions in most samples were (in order of decreasing concentration) P associated with $CaCO_3$, residual organic P, associated with Fe and Al hydrous oxides, and P in the fulvic acid fraction. Concentrations of P in each fraction were followed at 3-wk intervals from May to October and on some additional dates at two or more sites. There were only two systematic changes in concentration over this time. The amount of P incorporated in the sedimentary biomass increased during the summer, probably at a time of high microbiological activity; this was followed by a sharp decrease and another slower increase in late summer. Available P steadily increased during the growing season, but returned to near the previous year's value 12 mo later. Dissolved P in water adjacent to the sediments showed a peak in concentration corresponding to the decrease in biomass P in midsummer. The results are consistent with a picture of P cycling in which forms of the element are first incorporated in the biomass, but are later released to the water column or to available sites on the sediment upon decomposition of the biomass.

On a mesuré la distribution du phosphore dans les sédiments de la baie de Quinte. Les principales fractions dans la plupart des échantillons étaient (dans l'ordre décroissant de concentration) le P associé au $CaCO_3$, le P organique résiduel, le P associé aux oxydes de Fe et d'Al hydratés et le P dans la fraction de l'acide fulvique. La concentration du P dans chacune des fractions a été mesurée à intervalles 3 sem entre mai et octobre ainsi qu'à certaines autres dates à deux ou plusieurs emplacements. Au cours de cet intervalle, il ne s'est produit que deux changements systématiques de concentration. La quantité de P incorporée dans la biomasse sédimentaire s'est accrue au cours de l'été, probablement au cours d'une période d'intense activité microbiologique; cette phase a été suivie d'une baisse prononcée et d'une nouvelle augmentation plus lente à la fin de l'été. La teneur en P disponible s'est régulièrement accrue durant la saison de croissance, mais au bout de 12 mo, elle était pratiquement revenue à sa valeur de l'année précédente. Le P dissous dans la tranche d'eau adjacente aux sédiments a atteint un pic de concentration qui correspond à la diminution du P dans la biomasse au milieu de l'été. Les résultats obtenus sont conformes à la notion d'un cycle du P qui, sous ses premières formes, est incorporé dans la biomasse, mais est ensuite remis en circulation dans la colonne d'eau ou peut se fixer sur des sites libres dans les sédiments lors de la décomposition de la biomasse.

Received October 7, 1991
Accepted July 8, 1992
(JB252)

Reçu le 7 octobre 1991
Accepté le 8 juillet 1992

Phosphorus chemistry studies in lake sediments are of importance because this element is a major factor in the excessive and deleterious fertilization of lakes (Vollenweider 1968; Lee 1970). Syers et al. (1973) described a scheme that represents the general forms and interactions of P in lakes in terms of the relationships between the major P compartments of a lake system. Because the different P compartments are in dynamic equilibrium with one another, any change in concentration of P in one compartment may affect the concentration of P in the others. Of particular interest in the present study is the partitioning of P associated with sedimentary material into labile and nonlabile forms and the timing of its release to the water column. Such "internal loading" could contribute adversely to increased soluble P concentrations, even when external controls of the element were being imposed to reduce eutrophication.

The Bay of Quinte (BOQ) is a long and narrow embayment in the northeastern part of Lake Ontario (Johnson and Hurley 1986); it is 64 km long and 254 km^2 in area (Fig. 1). The upper Bay, running west to east, is shallow and well mixed with little or no temperature or dissolved O_2 stratification during the summer season. The middle (northeast to southwest) and lower BOQ (approximately west to east) become increasingly deeper and develop a distinct thermocline. The lack of vertical mixing in these deeper parts of the Bay results in low levels of dissolved O_2 near the sediment surface.

According to Bangay (1981), the Bay was already showing early signs of eutrophication in the late 1880's, although Warwick (1978) claimed that it could still be described as oligotrophic. Since then, increasing population and changing settlement patterns around the Bay have affected the trophic state. More than 50% of the population of the townships with frontage on the water is contained in five municipalities (Trenton, Belleville, Deseronto, Napanee, and Picton; total population approximately 100 000), and because of the urban concentration, aquatic P levels have been strongly influenced by point-source discharge systems (Sly 1986). Although a change from dispersed septic systems to centralized treatment began about 1890, an increase in nutrient loading to the Bay may actually have occurred then (Sly 1986). This is because the municipal facilities provided only primary treatment, with little control on P effluents. Furthermore, the introduction of high-phosphate

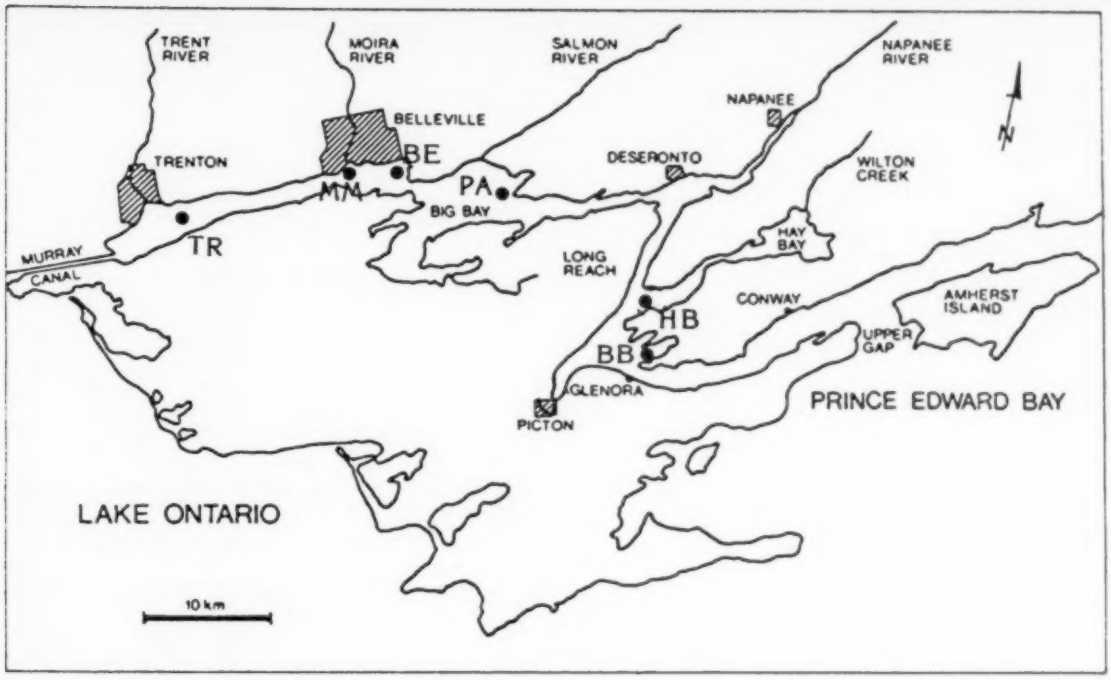

FIG. 1. Bay of Quinte, showing sampling sites.

detergents in the 1940's undoubtedly also increased nutrient loading (Johnson and Owen 1970). In the period from 1973 onward, major advances in point-source P control were introduced and there was early but short-lived improvement in BOQ water quality. These improvements in water quality were not maintained and it was appropriate to invoke the Cusp Catastrophe model of eutrophication to explain trends in the trophic state of the Bay, evident in the early 1980's (Minns et al. 1986). In this model, internal P loading is a very important factor.

It is now believed that years of excessive external P loading have resulted in high accumulation of P in the sediment (Johnson and Hurley 1986), and the Bay is now undergoing a process of self-purification (Nürnberg 1991) during which P is released from the sediment to the water. The means by which P goes into solution is influenced by a number of factors, such as the chemical nature of the sediments, physical mixing, redox status, pH, temperature, and bioturbation (Mortimer 1941; Pomeroy 1960; Rigler 1964; Olsen 1966).

Of particular interest in this study are the chemical forms of the P in the sediments of the Bay. Sediment P has usually been considered to exist in two main forms: organic P (P_o) and inorganic P (P_i). Operationally, each of these may be further divided into a number of subfractions according to the analytical scheme used. Depending on circumstances, components of both the P_o and P_i fractions of the element may be responsible for high P release rates in summer, and the release may involve both biotic and abiotic factors.

Using the model proposed by Syers et al. (1973), the potential problem of internal loading (release of sedimentary P to the overlying water column) can be framed into two questions: (1) Which P species are present in the particulate form in the sediments and with which organic component or mineral species in the sediment are they associated? (2) What fraction of the sediment P is potentially labile and what is its interaction with the "not-so-labile" forms?

The present study examines changes in the various P fractions at several sites in the upper and middle BOQ during a single summer season (with an additional measurement 1 yr later) in an attempt to identify which forms are labile. Such information could lead to a better understanding of the processes contributing to internal loading.

Materials and Methods

Sediment Sampling

Sediment core samples were collected at water depths of 2.5–6 m from six sites: Hay Bay (HB), Byggot Bay (BB), Point Anne (PA), Belleville (BE), the mouth of the Moira River (MM), and Trenton (TR) (Fig. 1). Samples were collected in mid-February and October 1988 at all sites except BB; further samples were taken at approximately 3-wk intervals at HB and BB from May to October 1988 with an additional sample in July 1989. Samples at HB and BB were obtained at depths of 4 and 2.5 m, respectively.

At each site, three sediment samples were collected from within a 10-m radius using a Benthos gravity core sampler (Benthos Inc., Edgerton Drive, North Falmouth, MA 02556, USA) and these were divided into two sections each, consisting of the top 10-cm layer (0–10 cm) and the bottom layer (>10 cm). Because the top 6–10 cm of sediment was very fluid, this material appeared to be well mixed in situ and was probably further mixed during collection and extrusion. For this reason, it was not appropriate to subdivide the cores just below the sediment–water interface into sections of less than 10 cm. The three samples were combined to make a composite representative of the site.

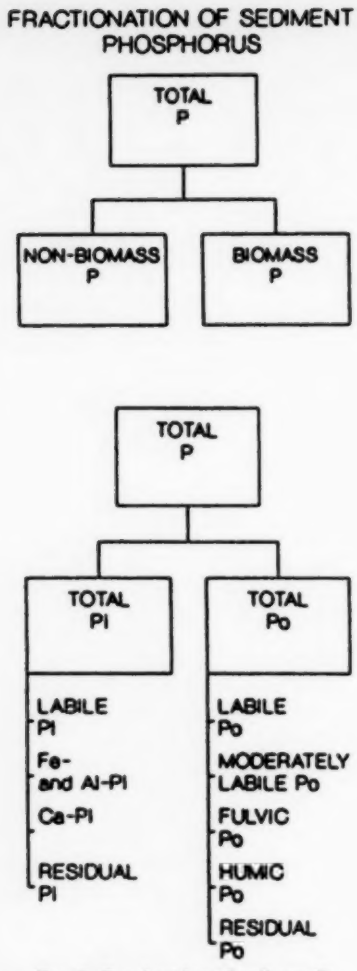

FIG. 2. Fractionation of sediment P.

The sediments were cleaned of debris by handpicking, wet-sieved to remove large pieces of plant material and living biomass, and stored at 4°C prior to analysis. Wet samples were used for all analyses, although results are reported on an oven-dry (100°C) basis.

Sediment Analysis

All analyses were done on duplicate portions of sediment. After extraction and or dissolution of subsamples, the P was determined by the method of Murphy and Riley (1962). The detection limit on the final solution was 3 $\mu g \cdot L^{-1}$ and that for the sediment depended on the dilution(s) carried out in each analysis.

More than one fractionation scheme was used for the analysis. Some of the procedures are not operationally exclusive, so that some forms of P occur in more than one fraction. Figure 2 shows the fractions which have been determined.

Five subsamples were required for a complete analysis.

Subsample 1

Total P (TP) was determined after dissolving 1.5 g of sediment in $HNO_3/HClO_4/HF$ and taking it up in 50 mL of water. In this and in all other cases, appropriate further dilution was required before analysis (Page et al. 1982).

Subsample 2

Organic matter (OM) was determined by ignition of a 2.5-g subsample at 500°C for approximately 5 h with weighing before and after. The ignited sample was subsequently extracted with 1.0 M H_2SO_4 for 3 h to obtain a value for total extracted P (D-TP) which could be used along with a value obtained on subsample 3 in order to obtain the concentration of total organic P ($D\text{-}TP_o$).

Subsample 3

A 2.5-g portion of sediment was extracted with 30 mL of 1.0 M H_2SO_4 for 3 h. This extract was analyzed to give a concentration of total inorganic P ($D\text{-}TP_i$). $D\text{-}TP_o$ by the ignition method was then calculated (Page et al. 1982) as

$$D\text{-}TP_o = D\text{-}TP - D\text{-}TP_i.$$

The unignited, H_2SO_4-extracted sample was further extracted in sequence to give three fractions as follows. Using 150 mL of 0.5 M NaOH, the sediment was extracted for 6 h. An aliquot of this extract was digested with HNO_3 and $HClO_4$ and analyzed to yield the concentration of humic plus fulvic P_o. Another aliquot of the extract was acidified with HCl to a pH of 1.0–1.8 to precipitate humic acids, filtered, and the filtrate digested as above. Analysis of this solution gave a value for fulvic P_o. Subtracting this value from that for humic plus fulvic P_o. Subtracting this value from that for humic plus fulvic P_o yielded the concentration of humic P_o. The residue from the NaOH extraction was then ignited at 500°C and subsequently extracted with 1.0 M H_2SO_4 in order to determine the residual P_o concentration of the sediment. Residual P_i was then determined by dissolution of the residue from the P_o determinations in $HNO_3/HClO_4/HF$. Rationale and details of these procedures are described in Oluyedun et al. (1991).

Subsample 4

A 2.5-g portion of sediment was extracted with 40 mL of 1.0 M NH_4Cl (pH 7.0) for 2 h. Concentrations of P in this extract were always near or below the detection limit and are not reported in this paper. The residue was then extracted with 40 mL of 0.1 M NaOH for 17 h, and after appropriate dilution the solution was analyzed to give a concentration of P associated with the hydrous oxides of Fe ($Fe\text{-}P_i$) and Al ($Al\text{-}P_i$). The P associated with $CaCO_3$ ($Ca\text{-}P_i$) was subsequently determined after extraction of the residue with 0.5 M HCl. Details of these procedures are given in Hieltjes and Lijklema (1980).

Subsample 5

Biomass P was determined by a method which has been developed for application to soils (Brookes et al. 1982; Hedley and Stewart 1982; McLaughlin et al. 1986). Analysis for these fractions was initiated not more than 1 h after sample collection. Three 2.5-g subsamples were taken. Subsample 5a was extracted with 20 mL of 0.5 M $NaHCO_3$ (pH 8.5) for 30 min, subsample 5b was fumigated with hexanol for 36 h and then extracted with the aqueous $NaHCO_3$ solution, and subsample 5c was extracted with the same reagent after the sediment had been spiked with P_i (as an aqueous solution of KH_2PO_4) equivalent to 25 $\mu g \cdot g$ oven-dry soil^{-1}. The microbial biomass P content of the soil was calculated by

$$\frac{25\,(C_b - C_a)}{0.4\,(C_c - C_a)}\ \mu g\ P \cdot g\ \text{oven-dry soil}^{-1}$$

where C_e, C_b, and C_c refer t$_o$ the concentrations in the extracts of subsamples 5a, 5b, and 5c, respectively.

The concentration obtained after direct extraction of subsample 5a was a measure of labile P_i. A portion of this solution was digested with HNO_3 and $HClO_4$ to give total labile P, and the difference between this concentration and that of labile P_i was the labile P_o.

Water Samples and Analysis

Dissolved oxygen (DO) in lake water was measured in the field using a Bausch and Lomb Spectrokit O_2 probe, after calibration with the manufacturer's standards. pH and water temperature were also measured directly in the field.

Water samples were collected at approximately 2- to 3-wk intervals between mid-June and early October using polymethylmethacylate dialysis chambers (approximately 75 mL) fitted with 0.45-μm Millipore filters at one end and protected by perforated screw caps. These chambers were filled with distilled water and placed in a larger transport container, also filled with distilled water. The chambers were purged by bubbling N_2 gas through the large container to remove DO. At the sampling site, they were placed in an array which was lowered to the sediment surface so that one pair faced upwards (as in a sediment trap), another pair faced downwards, and the last pair was arranged horizontally (exposed to ambient water). All of the chambers were situated approximately 10 cm above the sediment surface. The arrays were allowed to equilibrate with the ambient lake water for the entire period (approximately 3 wk) between sampling. Further details concerning the sampling apparatus and technique are available in Sly (1990).

P analysis was carried out within 24 h of obtaining samples. In all cases, P in water samples was determined using the AOAC (1984) procedure. The detection limit was 3 μg $P{\cdot}L^{-1}$ in lake water.

Results and Discussion

For the sediments examined, the content of OM was 22 ± 6%, with the exception of the TR sediment (7%). For most fractions at all stations, the TP concentrations were higher in the top layer (0–10 cm) than in the bottom layer (>10 cm). However, the relative concentrations of P fractions in the core samples were similar at all depths, and the discussion which follows is based on results for the 0- to 10-cm layer unless otherwise noted.

TP, TP_i, and TP_o

TP values ranged from 1.0 to 1.8 mg P·g dry weight of sediment^{-1} with 60–80% as inorganic TP_i (Table 1). All the fractions, Fe–P_i, Al-P_i, residual P_i, humic P_o, fulvic P_o, and residual P_o, were measured separately, and the sum of these fractions should equal the value of TP. Concentrations of TP_i and TP_o derived from the ignition method were similar to the sum of appropriate P fractions. The sum of the concentrations of TP_i and TP_o also yielded results which were similar to the TP values obtained by direct $HNO_3/HClO_4/HF$ dissolution (Table 1).

Concentrations of Individual P Fractions

Almost half of the TP in the BOQ sediment was found to be in the fraction associated with $CaCO_3$ (Ca-P_i, Table 1), reflecting the calcareous nature of the sedimentary material. The relative concentrations of Fe-P_i and Al-P_i were much lower. The residual P_i concentrations were small at all stations, rarely exceeding 3 μg $P{\cdot}g^{-1}$ (less than 1% of TP_i). In soils, however, it has been reported that the residual P_i may comprise as much as 70 μg $P{\cdot}g^{-1}$ or 5.5% of the TP_i (Williams et al. 1967). It is expected that this fraction includes P associated with resistant primary minerals.

Except for stations BB and TR, the P_o fraction having the highest concentration was residual P_o; at those two stations, the fulvic P_o fraction was larger (Table 1). Labile P_o concentrations for all samples were small and rarely exceeded 6 μg $P{\cdot}g^{-1}$.

Variation in P Fractions over a 1-yr Period

Individual inorganic P fractions, with the exception of labile P_i, are relatively constant and do not show any systematic variation throughout the sampling period (Fig. 3). Likewise, the concentrations of fractions of P_o remained generally constant over the year.

Biomass P and Labile P_i

Results for biomass P concentrations at five stations on February 19, 1988, showed that concentrations were always substantially higher in the top layer of sediment (Table 2). Also, it was noted that during periods of high concentrations of biomass P in the sediment, the difference in the concentrations of biomass P in the top and the bottom layers became larger. This observation is consistent with the presence of microorganisms concentrated in the top layer of the sediment.

Biomass P and labile P_i were the only fractions that showed systematically large variations over the 1-yr period (Fig. 4). The biomass P at station HB rose from a value of 34 μg $P{\cdot}g^{-1}$ on February 19 to a peak value of 194 μg $P{\cdot}g^{-1}$ on June 14 and stayed near this value to July 5 before falling sharply to 31 μg $P{\cdot}g^{-1}$ on July 22. A second, smaller peak was observed in the results of the sample collected on September 12.

The labile P_i at station HB rose from 4 μg $P{\cdot}g^{-1}$ on February 19 to 97 μg $P{\cdot}g^{-1}$ on October 6. By July 22, the labile P_i concentrations had exceeded those of biomass P. Concentrations of labile P would not be expected to rise indefinitely, and it was thought that high levels of labile P_i would likely drop over the winter period before rising again during summer of the following year. To evaluate this hypothesis, another sampling trip was made on July 12, 1989, and it was found that the labile P_i concentration had decreased to 30 μg $P{\cdot}g^{-1}$ which is near the value found at the same time 1 yr previously.

The results obtained with samples collected at station BB were comparable. The peak concentration for biomass P at this station occurred on June 14 (112 μg $P{\cdot}g^{-1}$); after this the concentration dropped gradually to 27 μg $P{\cdot}g^{-1}$) by August 4. There was also a second peak on September 12, as had been observed at station HB. Likewise, at BB the labile P_i values more than doubled over the growing season, although in this case the increase was not continuous as in HB.

The implication of these results may be significant with respect to understanding eutrophication in the BOQ. By analogy to the situation with soils, labile P_i is believed to represent the form of P most readily available to phytoplankton and aquatic macrophytes; this form may also have significant solubility in the ambient water. Since the concentration of labile P_i increases considerably near the time that biomass P levels are declining, the results are consistent with the hypothesis that P is incorporated in the sedimentary biomass during the summer season; when dead microorganisms decompose, most of the P

TABLE 1. Mean P concentrations in $\mu g \cdot g^{-1}$ (and standard deviations) in various fractions for all stations (0–10 cm). D-TP_i = determination of TP_i by ignition method; D-TP_o = determination of TP_o by ignition method; D-TP = determination of TP by ignition method; TP = TP_i + TP_o.

		Station					
	Date	TR	MM	BE	PA	HB	BB
Fe-P_i and Al-P_i	19/2/88	26 (0.7)	150 (5.5)	120 (3.0)	153 (3.3)	125 (2.8)	
	6/10/88	60 (0.6)	109 (1.0)	139 (1.7)	170 (2.0)	113 (0.8)	52 (0.5)
Ca-P_i	19/2/88	817 (5.0)	899 (5.2)	976 (4.2)	744 (2.1)	578 (3.5)	
	6/10/88	800 (3.9)	1050 (5.6)	847 (3.0)	758 (2.7)	581 (2.9)	932 (3.9)
Residual P_i	19/2/88	0.4 (0.1)	1.5 (0.1)	1.0 (0.1)	1.7 (0.1)	0.3 (0.1)	
	6/10/88	0.5 (0.1)	0.8 (0.1)	1.0 (0.2)	0.7 (0.1)	1.4 (0.1)	3.0 (0.2)
Fulvic P	19/2/88	82 (1.1)	142 (2.2)	150 (0.8)	125 (1.5)	114 (1.9)	
	6/10/88	150 (1.5)	153 (2.5)	116 (1.1)	126 (1.6)	122 (2.2)	130 (2.0)
Humic P	19/2/88	53 (1.1)	62 (1.1)	60 (0.9)	80 (1.0)	105 (1.8)	
	6/10/88	63 (1.3)	120 (2.0)	115 (2.1)	110 (1.5)	61 (1.5)	40 (0.9)
Residual P_o	19/2/88	60 (0.9)	366 (2.2)	276 (2.5)	291 (1.9)	233 (2.5)	
	6/10/88	140 (2.0)	424 (3.2)	219 (3.0)	263 (2.1)	269 (2.9)	92 (1.2)
TP_i	19/2/88	843	1051	1097	899	703	
	6/10/88	861	1160	987	929	695	987
D-TP_i	19/2/88	802 (2.7)	1000 (5.5)	998 (3.7)	800 (3.5)	685 (3.9)	
	6/10/88	816 (3.0)	1123 (5.2)	957 (4.7)	920 (4.0)	659 (2.5)	956 (4.4)
TP_o	19/2/88	195	570	486	496	452	
	6/10/88	353	697	450	499	452	262
D-TP_o	19/2/88	200 (1.5)	532 (3.8)	475 (3.5)	500 (2.0)	460 (2.6)	
	6/10/88	324 (1.9)	655 (3.9)	439 (2.9)	489 (2.3)	425 (3.0)	274 (2.7)
TP	19/2/88	1038	1621	1583	1395	1155	
	6/10/88	1214	1857	1437	1428	1147	1249
D-TP	19/2/88	1000 (5.6)	1600 (9.4)	1577 (8.7)	1284 (2.3)	1064 (4.0)	
	6/10/88	1212 (6.9)	1795 (9.5)	1378 (5.9)	1399 (4.7)	1163 (8.2)	1190 (6.9)
%TP_i	19/2/88	81	65	69	64	61	
	6/10/88	71	62	69	65	61	79
%TP_o	19/2/88	19	35	31	36	39	
	6/10/88	29	38	31	35	39	21

associated with the cellular material is initially released into the labile P_i fraction. The decline in labile P_i that may have occurred during the winter season further suggests that, in the longer term, labile P_i is lost to the water or to plants, or reverts to a more stable form.

A low concentration of DO in the water columns above the bed was found at stations HB and BB on August 4 (Fig. 5), a date just following the large decrease in biomass P (Fig. 4). This suggests that microorganisms grew in the early summer and then died; the subsequent decomposition was accompanied by a depletion of O_2. It was at this time that labile P_i values for both stations began to exceed those of biomass P values.

Chlorophyll *a* values obtained from the Ministry of Environment (F. Stride, pers. comm.) for station HB between May 4 and October 23 showed that the highest value was recorded in early August. This is consistent with the assumption that there was an abundant supply of P for algal growth during that period.

It is important to note that the biomass P measurements reported above are purely chemical and no information may be inferred about the nature of the microorganisms in which the P accumulates. Such organisms may settle to the sediment from the water above and/or grow within the sediment itself.

The pattern noted for biomass P and labile P_i lends support to hypotheses that biotic processes, as well as abiotic factors (e.g. dissolution of FeOOH–PO_4 complexes, pH, temperature, etc.), affect release of P from sediment to the waterbody.

It is difficult to assess the relative significance of biotic versus abiotic P release, as their rates are temperature controlled and both types of release would occur at the same time and under the same conditions. As the microorganisms begin to die, they use up DO in the water, thereby creating anoxic conditions at the bottom of the lake; this is the condition needed for the abiotic dissolution of FeOOH–PO_4 complexes. Simultaneously, P within the cells of microorganisms could be released into the water column or could become associated with the labile P_i fraction.

In a recent study, Gächter et al. (1988) found that the release of "soluble reactive phosphorus" (SRP) by bacteria and the dissolution of FeOOH occurred at nearly the same redox potential. This observation was used as an explanation as to why direct microbial contributions to uptake and release of P have been overlooked and only the very convincing abiotic model has been generally accepted. They found that there was a time lag between the beginning of the dissolution of the FeOOH–PO_4 complex and release of P from sediment and inferred that the P originated from the biomass.

Analysis of P in Water

Mean values and standard deviations for SRP and dissolved P (dP) in the dialysis chambers showed that P concentrations in the horizontal chambers were always less than in both the

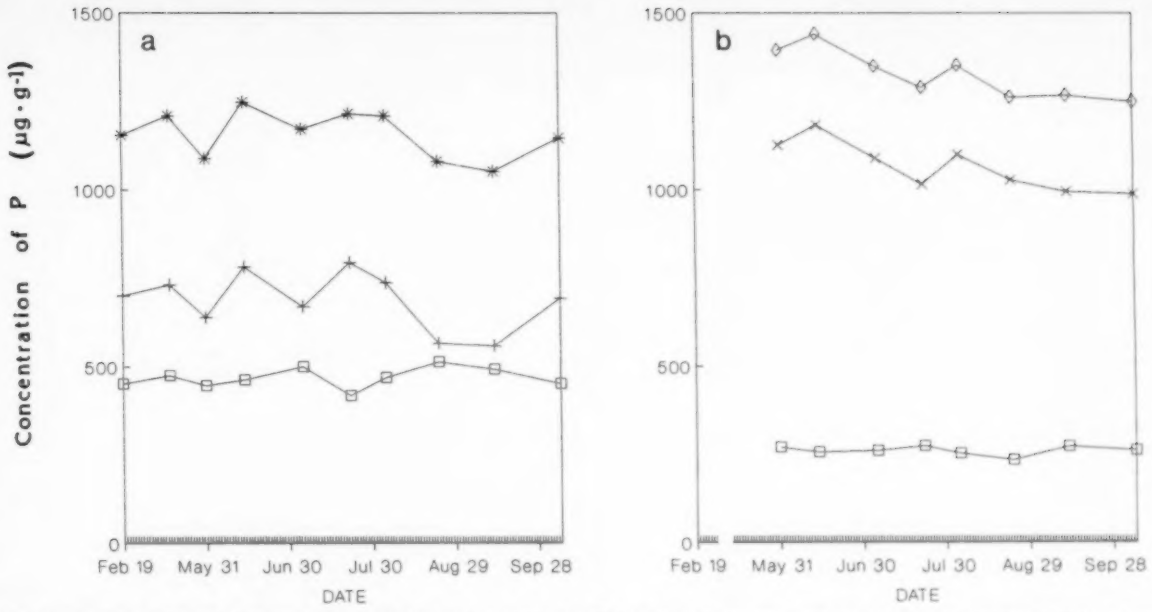

FIG. 3. Temporal variation in concentration of TP(*), TP_o(+), and TP_i (□) in (a) Hay Bay sediment and (b) Byggot Bay sediment (0–10 cm).

TABLE 2. Biomass P, labile P_i, and labile P_o concentrations in $\mu g \cdot g^{-1}$ (and standard deviations) for five stations on February 19, 1988.

Station	Depth (cm)	Biomass P	Labile P_i	Labile P_o
TR	0–10	18.1 (1.2)	3.2 (0.3)	2.1 (0.2)
	>10	8.4 (0.9)	1.2 (0.1)	1.4 (0.1)
MM	0–10	74.4 (2.8)	13.0 (1.7)	0.3 (0.1)
	>10	44.8 (1.9)	12.6 (1.6)	0.3 (0.1)
BE	0–10	50.0 (2.5)	14.5 (1.9)	1.5 (0.1)
	>10	29.7 (1.5)	6.9 (0.9)	1.2 (0.1)
PA	0–10	46.2 (2.2)	10.6 (1.9)	2.7 (0.2)
	>10	25.3 (1.0)	8.6 (1.7)	1.0 (0.2)
HB	0–10	33.9 (1.3)	4.1 (0.5)	6.0 (0.7)
	>10	27.1 (1.3)	0.4 (0.1)	7.0 (0.9)

up- and down-facing chambers (Table 3). This is consistent with data obtained by Sly (1990) from samples taken in other parts of the BOQ in 1987. Sly (1990) suggested that the horizontal chamber value characterized the ambient P concentration near the base of the water columns, while the up chambers showed potential nutrient release from the degradation of settling particulates (largely organic material) and the down chambers were influenced by release of P from the partly aged sediment. Using this reasoning, the current results give evidence that nutrient release, both from freshly deposited material and from existing sediments, contributes significantly to the P concentration near the sediment surface. In all cases the SRP made up approximately 60% of the dP value.

One purpose of analyzing P in water samples was to determine whether there was a trend in which dP in the waterbody showed a temporal maximum, most likely during a period when P was released from freshly deposited or aged sediment biomass by biological decomposition or by chemical processes associated with the low redox status accompanying the decay. Individual dP values fluctuated between 10 and 100 $\mu g \cdot L^{-1}$, and the mean values (for all chambers) of samples collected on the same day showed a concentration peak on August 4 at both stations (38 $\mu g \cdot L^{-1}$) in HB and 43 $\mu g \cdot L^{-1}$ in BB). The mean values for other sampling dates at station HB varied only slightly, ranging from 20 to 26 $\mu g \cdot L^{-1}$, while in station BB, the variation in mean values was between 12 and 40 $\mu g \cdot L^{-1}$. The August 4 peak is consistent with the idea of biological release of P from the sediment in that the peak concentration in dP occurred on the sampling date corresponding to the large decrease in biomass P (Fig. 4). Results of analysis of the water samples obtained from another part of HB in 1987 (Sly 1990) also showed a similar maximum dP concentration in early August of that year. These observations do not preclude the possibility that there is some release of P throughout the year. The released P may go into the water column, or may be taken up by aquatic plants, or it may remain associated with the sediment as forms that are measured in the available fraction.

These results suggest that time-specific annual monitoring of the biomass P and the available P could be useful in assessing changes in internal P loading. Measurements of biomass P made in early July and/or early September would indicate accumulations of the element in this fraction, while a measurement of

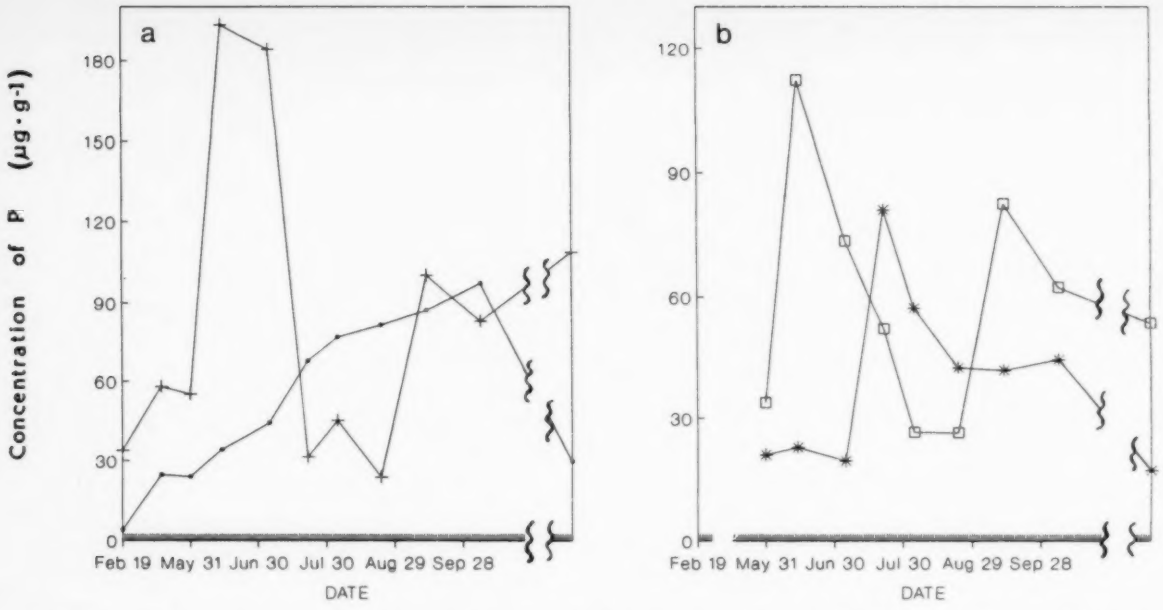

FIG. 4. Temporal variation of biomass P(□) and labile P(*) concentration in (a) Hay Bay sediment and (b) Byggot Bay sediment (0–10 cm).

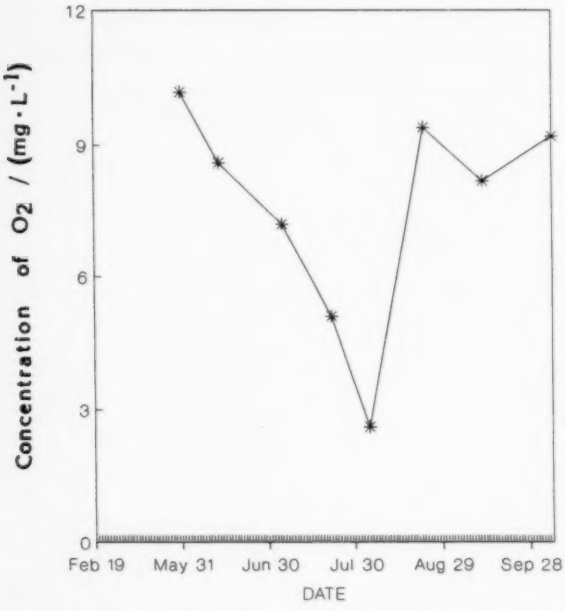

FIG. 5. Temporal variation in DO_2 at the sediment surface in Hay Bay.

available P in the September sample would be useful for assessing the amount of P which is likely to be released into the water column during the winter season. Decisions regarding the best time for such sample collection would depend on detailed knowledge of the biological growth cycles in the BOQ, and exact dates might vary from year to year.

TABLE 3. Mean concentrations in $\mu g \cdot L^{-1}$ (and standard deviations) of dP and SRP throughout the growing season at HB and BB in three differently oriented chambers.

	HB		BB	
Chambers	dP	SRP	dP	SRP
Up-facing	35 (16)	21 (13)	27 (17)	22 (14)
Down-facing	24 (7)	15 (8)	39 (28)	23 (8)
Horizontal	20 (7)	11 (6)	19 (8)	12 (8)

Conclusion

It was found that, amongst the fractions of sedimentary P, concentrations of the biomass P and labile P_i fractions changed significantly during the sampling period, while other determined fractions of P_i and P_o remained constant. Concentrations of biomass P were higher in the top layer of the sediment than in the subsurface layer, probably reflecting the fact that microorganisms were concentrated near the sediment surface. It is not clear from this study whether these were living in the sediment itself or had settled out of the water column. There were two temporal peaks of biomass P concentration in the sediment in early and late summer which corresponded to maximum chlorophyll *a* concentrations.

It was also observed that there was a consistent increase in labile P_i during the summer suggesting that some of the biomass P later became associated with the labile P_i fraction. This fraction can be expected to be readily accessible for lake enrichment. The DO concentrations support the observation based on biomass P values that dead microorganisms were decomposing after a period of accelerated growth.

A peak in dP in water just above the sediment surface occurred at the time immediately following the drop in biomass P concentration in midsummer, indicating that some of the P was also released directly to the water column.

In general, the observations made in this study are consistent with the hypothesis that there is a microbial contribution to P release from sediments in addition to P release by purely physicochemical processes. However, it is not yet possible to separate the contribution of microorganisms to the fixation or production of phosphate from the abiotic coupling between Fe and P cycles.

Acknowledgments

Financial and technical support from Queen's University, the Canadian International Development Agency, and the Ministry of the Environment (Government of Ontario) is gratefully acknowledged.

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Branchial Morphological and Endocrine Responses of Rainbow Trout (*Oncorhynchus mykiss*) to a Long-Term Sublethal Acid Exposure In Which Acclimation Did Not Occur

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Audet, C., and C. M. Wood. 1993. Branchial morphological and endocrine responses of rainbow trout (*Oncorhynchus mykiss*) to a long-term sublethal acid exposure in which acclimation did not occur. Can. J. Fish. Aquat. Sci. 50: 198–209.

Changes in branchial morphology and in plasma cortisol, adrenaline, and noradrenaline were quantified throughout an 81-d exposure of rainbow trout (*Oncorhynchus mykiss*) to sublethal acidity (pH 4.8) in artificial soft water and after a 5-h acid challenge (pH 4.0) of naive fish and 81-d acid-preexposed fish. Changes in branchial morphology at pH 4.8 were generally very mild and characterized by slight increases in filamental mucous cells and decreases in lamellar mucous cells. Chloride cell numbers and branchial Na^+–K^+- and total ATPase activities did not change. The filamental epithelium thickened, but the water–blood diffusion distance in the lamellae decreased during chronic exposure. Cortisol was significantly elevated throughout whereas catecholamines exhibited relatively little response. Response to acute pH 4.0 challenge was similar in naive and 81-d acid-exposed fish: epithelial damage, increase in visible mucous cells, loss of chloride cells by necrosis, and high cortisol levels but no changes in lamellar or filamental epithelial thickness, diffusion distance, ATPase activities, or catecholamine levels. Previously reported physiological data from these same trout demonstrated that sensitization rather than acclimation had occurred. Therefore, these observations support the view that acclimation does not occur in the absence of significant branchial damage and repair.

Les changements morphologiques au niveau des branchies ainsi que les variations de cortisol, d'adrénaline et de noradrénaline plasmatiques ont été quantifiés chez la truite arc-en-ciel (*Oncorhynchus mykiss*) au cours d'une exposition de 81 jours à pH acide sous-létal (pH 4,8) dans une eau à faible teneur ionique ainsi qu'après une exposition de 5 h à pH 4,0, chez des poissons n'ayant jamais été exposés à l'acidité et chez des poissons préexposés à l'acidité durant 81 jours. Les changements morphologiques au niveau des branchies étaient généralement faibles et caractérisés par une légère augmentation des cellules à mucus sur les filaments branchiaux et une diminution sur les lamelles branchiales. Nous n'avons observé aucun changement du nombre de cellules à chlorure, ni de l'activité Na^+–K^+ et ATPasique totale branchiale. L'épaisseur des filaments a augmenté mais la distance de diffusion lamellaire a décru au cours de l'exposition chronique. Le cortisol est resté significativement plus élevé durant toute la période d'exposition alors qu'il n'y a eu que très peu de réponse au niveau des catécholamines. La réponse à pH 4,0 a été similaire chez les poissons pré-exposés pour 81 jours et chez ceux non pré-exposés à l'acidité: dommages épithéliaux, augmentation du nombre visible de cellules à mucus, perte de cellules à chlorure par nécrose, concentrations plus élevées de cortisol mais aucun changement significatif au niveau de l'épaisseur des épithéliums lamellaires et filamentaux, de la distance de diffusion, des activités ATPasiques ou des niveaux de catécholamines. Des données précédemment publiées et provenant des mêmes poissons indiquaient une sensibilisation plutôt qu'une acclimatation. Les présentes observations supportent donc l'hypothèse que l'acclimatation ne se produit pas en l'absence de dommages branchiaux significatifs et de réparation subséquente.

Received October 25, 1991
Accepted July 8, 1992
(JB280)

Reçu le 25 octobre 1991
Accepté le 8 juillet 1992

The present investigation is part of an overall study on the ionoregulatory, endocrine, and branchial morphological responses of adult rainbow trout (*Oncorhynchus mykiss*) to chronic acid exposure (81 d) at pH 4.8 in artificial soft water (ASW) and the effects of such an exposure on the responses to a further acute acid stress (5 h at pH 4.0). Particular care was taken to duplicate the water chemistry characteristic of typical acid-threatened softwaters in eastern North America and to measure the various ionoregulatory, morphological, and endocrine parameters on the same individuals. The first part of our study (Audet et al. 1988) showed that chronic acid exposure produced a reduction of branchial influx (J_{in}) and efflux rates (J_{out}) and a negative net flux (J_{net}) of the two major plasma electrolytes (Na^+ and Cl^-). On a long-term basis, J_{net} values were restored to approximately control levels but plasma Na^+ and Cl^- concentrations remained depressed, glucose remained elevated, and hematological parameters remained disturbed. Thus, there was stabilization at a new steady state, but no recovery. The second part (Audet and Wood 1988) demonstrated that these chronically exposed fish suffered more extreme Na^+ and

Cl^- losses and other physiological disturbances than did naive fish when challenged with a more severe acid stress. Thus, there was sensitization rather than acclimation.

The first objective of the present work was to quantitatively characterize possible morphological and enzymatic alterations in the gills. Our immediate goal was to see whether or not these correlated with observed changes in branchial Na^+ and Cl^- fluxes and internal physiology during chronic and acute acid exposures. We also measured primary endocrine stress indicators (plasma cortisol and catecholamines) to better understand their role in the observed responses. Both agents have been implicated in the control of branchial transport function (Wood 1991) and cortisol in the control of branchial morphology (Perry and Wood 1985; Laurent and Perry 1990). We were also particularly interested in whether a sublethal exposure which did not induce either recovery or acclimation was associated with significant histopathological damage.

Recently, McDonald and Wood (1992) have proposed that branchial mechanisms of acclimation are a function of damage repair. By this hypothesis, for a toxicant to induce acclimation, it must first cause significant structural damage to the gill epithelium. While such damage is traditionally seen as a correlate of acutely lethal acid stress (Mallatt 1985; McDonald et al. 1991), several recent studies have suggested that chronic sublethal exposure to pure acidity, without significant involvement of metals, may cause little or no disruption of gill morphology and histology in salmonids (Jagoe and Haines 1983; Karlsson-Norrgren et al. 1986; Evans et al. 1988; Mueller et al. 1991). Other chronic studies do not agree (Daye and Garside 1976; Tietge et al. 1988; Laurent and Perry 1991). However, none of these investigations examined whether acclimation occurred. The present study, in combination with Audet et al. (1988) and Audet and Wood (1988), is therefore the first to assess gill morphological responses and to test for acclimation simultaneously.

Methods

Exposure and Sampling

This study was performed concurrently with those reported in two previously published papers (Audet et al. 1988; Audet and Wood 1988) which provide complete information on fish holding and experimental protocols. Key details are repeated here. The whole study was conducted in flowing artificial soft water to which the trout had been acclimated for 2–4.5 mo prior to testing. The study was divided into two main parts: long-term sublethal acid exposure (81 d at pH 4.8) and acute severe acid stress (5 h at pH 4.0), hereafter described as "acid challenge." Acid challenge was imposed either on fish kept under circumneutral conditions (pH ≈ 6.5, "naive" fish) or on fish which had been previously exposed to long-term sublethal acid conditions (3 mo at pH 4.8, "acid-preexposed fish"). Adult rainbow trout (200–300 g) of both sexes were obtained from a hardwater source (Spring Valley Trout Farm, Petersburg, Ontario) and initially held in Hamilton tap water (Ca^{2+} = 1800, Na^+ = 600, Cl^- = 800 $\mu eq \cdot L^{-1}$). Stock and experimental fish were kept at 15 ± 1°C under a 24 h light photoperiod. The fish were acclimated for 2–4.5 mo to ASW (Ca^{2+} = 50, Na^+ = 50, Cl^- = 100 $\mu eq \cdot L^{-1}$; pH 6.5) before being used as either controls (no acid exposure) or experimentals (chronic acid exposure or pH 4.0 challenge). None of the fish were in breeding condition. ASW production, fish density, feeding schedule, water acidification, and pH recording procedures are fully described in Audet et al. (1988).

The basic experimental protocol of 2–4.5 mo of ASW acclimation at pH ≈ 6.5 followed by up to 81 d of pH 4.8 exposure was repeated five times to yield the blood and tissue chemistry data reported by Audet et al. (1988). The morphological and endocrine results presented here for the long-term acid exposure come from three of these experiments starting in April, October, and January. Not all experimental times were sampled in each series, but control samples were taken at the start of every series. Simultaneous exposure of the fish to ASW at neutral pH was not run in parallel to each of the series in view of the long period allowed for initial acclimation to ASW in every experiment. As expected, given the constant temperature and photoperiod regimes, no significant differences among series were found in controls or at common sample times. Therefore the data from the different series were pooled. The pH 4.0 challenge experiments reported here were run at the end of the 81-d exposure which had been started in October, i.e. in early January. At this time, challenge exposures of naive and acid-preexposed fish were conducted *simultaneously*. Samples were taken from naive fish (pH 6.5) and acid-preexposed fish (pH = 4.8) both before and immediately after 5 h of exposure to pH 4.0.

In the 81-d sublethal acid exposure study, trout were sampled under control conditions (C) and at 1, 3, 8, 22, 50, and 81 d of exposure to pH = 4.8 and, in the challenge experiment, after 5 h of exposure to pH 4.0. At sampling, fish were individually anaesthetized in 0.01% MS-222 (pH adjusted to 6.5, 4.8, or 4.0 as appropriate with KOH). Blood was withdrawn immediately by caudal puncture and plasma obtained by centrifugation (10 000*g* for 2 min). An aliquot of plasma was immediately frozen at −80°C in liquid nitrogen for later determination of cortisol and catecholamines. After caudal puncture, the second gill arch of the left side of the fish was clamped at ventral and dorsal margins; the middle part was sectioned and put in 4% formaldehyde – 5% glutaraldehyde fixative solution buffered with sodium cacodylate (0.1 M, pH 7.2). A catheter was then introduced into the bulbus arteriosus and the gills were perfused with ammonium heparin (2500 $IU \cdot mL^{-1}$) diluted in Cortland saline solution (Wolf 1963) to "wash out" the red cells. The second gill arch of the right side was then sectioned and frozen in liquid nitrogen for later Na^+–K^+-ATPase activity determination.

Cortisol was measured by ^{125}I radioimmunoassay (New England Nuclear). Adrenaline and noradrenaline were determined on alumina-extracted plasma samples using high-performance (pressure) liquid chromatography (Waters 510 pump, reverse phase C-18 column) with electrochemical detection (Waters M460 ECP). All plasma hormone concentration determinations were in duplicate. For each fish, Na^+–K^+-ATPase activity was measured on gill homogenate by the method of Stagg and Shuttleworth (1982) and protein content by the method of Lowry et al. (1951). Average total gill ATPase activity and Na^+–K^+-ATPase activity are reported both per gram of gill tissue and per milligram of protein. Gill tissues were kept immersed in the fixative for a maximum period of 2 wk. They were then postfixed in 1% osmium, stained with uranyl acetate, dehydrated in ethanol, and embedded in Spurr. When embedded, tissues were oriented in order to obtain sagittal sections. Semithin sections (0.5 μm) were prepared and stained with toluidine blue. For controls and long-term sublethal acid-exposed fish (81 d), ultrathin (60–80 nm) sections were stained with lead citrate and chloride cells were examined in a Philips 300 electron microscope.

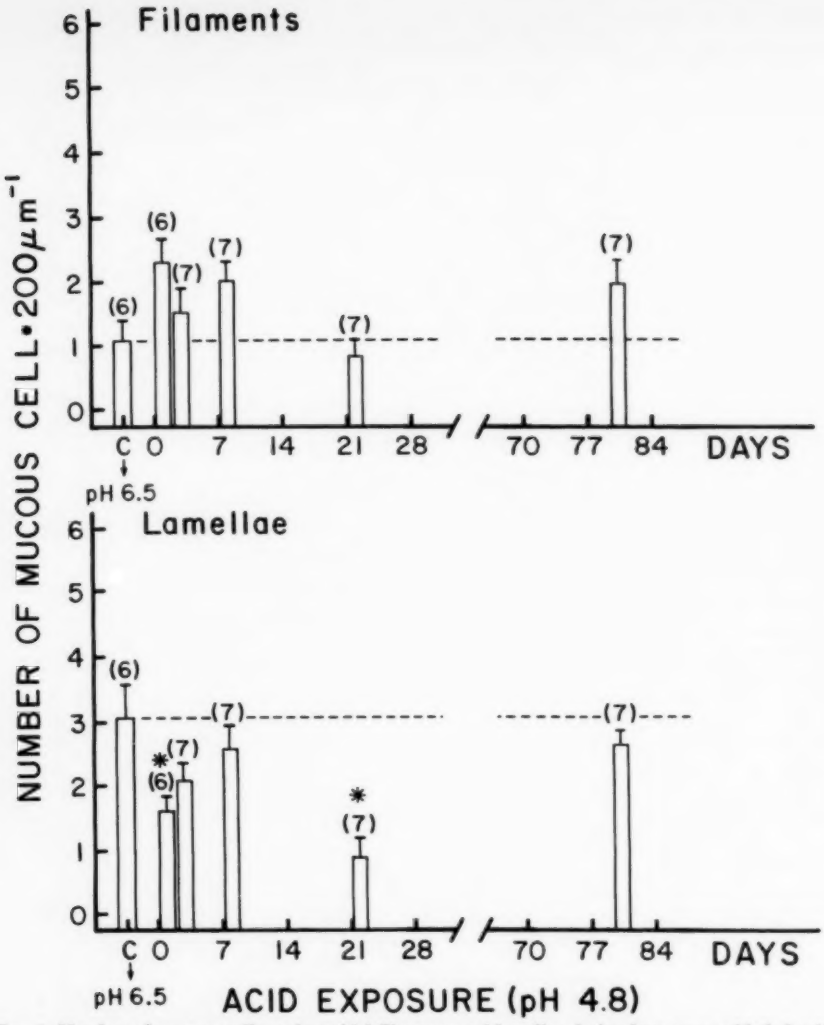

FIG. 1. Number of mucous cells on branchial filaments and lamellae during long-term sublethal acid exposure in rainbow trout. Means ± SEM; numbers of fish in parentheses. C = control ASW acclimated fish (pH 6.5). Asterisks represent significant differences from controls (level indicated by broken line). Results of the mean comparison tests are presented below: numbers represent the days of exposure, and single lines underscore days between which there was no significant difference:

Filaments:	22	C	3	81	8	1
Lamellae:	22	1	3	8	81	C

Histological Measurements

Histological measurements were done on a Leitz light microscope coupled with a Zeiss Videoplan image analysis system (MOP-40). A photomicrograph of a Merz grid was transferred onto a transparency which was fixed to the screen of the image analyzer. The system was calibrated using a stage micrometer (Leitz). The different parameters measured were interlamellar distance, thickness of filament, thickness of filamental epithelium, thickness of secondary lamellae, thickness of lamellar epithelium, water–blood diffusion distance, numbers of chloride and mucous cells on the filament, and numbers of chloride and mucous cells on the lamellae.

Interlamellar distance was measured at the base of lamellae on three different filaments per fish, employing 10 measurements per filament (total of 30 measurements per fish). For thickness of filament and secondary lamellae as well as thickness of their epithelia and diffusion distance, the points from which measurements were initiated were randomly chosen by the use of a Merz grid (Hughes and Perry 1976). Thickness of filament and thickness of filamental epithelium were determined on three filaments, with three measurements on each (total of nine measurements per fish). Thickness of secondary lamellae, thickness of lamellar epithelium, and water–blood diffusion distance were measured on 10 lamellae from three filaments per fish. As thickness of lamellar epithelium and

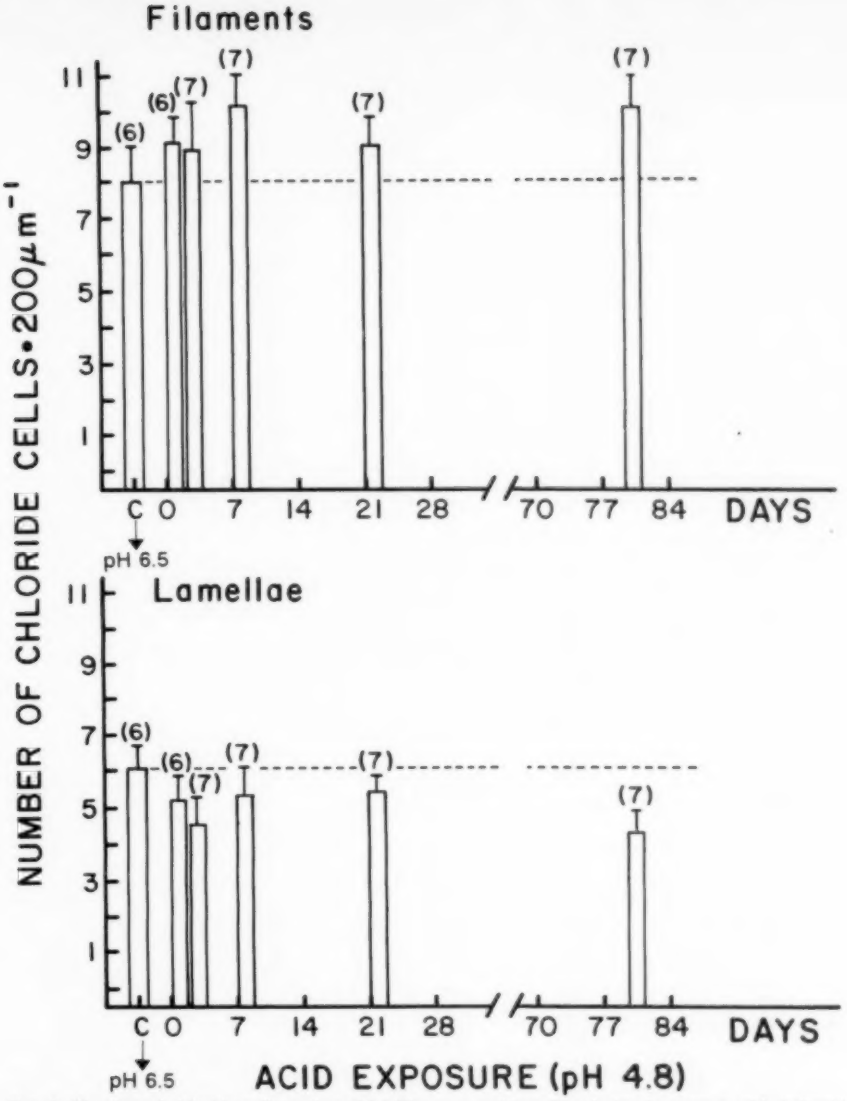

FIG. 2. Number of chloride cells on branchial filaments and lamellae during long-term sublethal acid exposure in rainbow trout. Differences not significant. See legend to Fig. 1 for other details.

water–blood diffusion distance were measured on high magnification (1125×), the field of our Merz grid did not cover the entire surface of a secondary lamella. Therefore, to ensure representative data for these two parameters, we took three different measurements per secondary lamella: one in the basal area, the second in the middle area, and the third one at the apical level. The points from which these measurements were initiated were randomly chosen by the use of the Merz grid. In total, thickness of the epithelium of secondary lamellae and water–blood diffusion distance were both obtained from 90 different measurements compared with 30 for thickness of secondary lamellae. Both mucous and chloride cells were counted on 10 secondary lamella from three filaments per fish. Length of the tissue sections covered was measured and the number of cells then reported per 200 μm. Each fish provided only one value for each parameter, this value being the average of all measurements of that parameter in that fish (9–90 depending on the parameter).

Statistics

Data have been routinely expressed as mean ± SEM (n) where n represents the number of fish. The data for long-term sublethal acid exposure were analyzed by nonbalanced one-way ANOVA, $p \leq 0.05$ (time of exposure tested as the source of variation), followed by a Tukey test of comparison of means adjusted for unequal n (T') (Sokal and Rohlf 1981) with $\alpha = 0.05$. All other statistical comparisons were done by t-test ($p \leq 0.05$). Data were tested for normality and for homogeneity of variances (Kolmogorov–Smirnov test and F_{max} test, respectively). All the data were normally distributed; however, in some cases, transformations were required to obtain homogeneity of

variances. Thus, data for cortisol and adrenaline were transformed as $\log_{10}(x)$ prior to ANOVA, while data for noradrenaline were transformed as $1/\sqrt{x}$.

Results

Long-Term Sublethal Acid Exposure

Sublethal acid exposure produced only slight changes in gill morphology. Except for chloride cell numbers, significant treatment effects (ANOVAs, $p < 0.05$) were observed on all measured parameters. However, these changes were often transient and/or of small magnitude.

Significant variations in the number of mucous cells were observed on both filaments and lamellae. The number of mucous cells tended to be higher at pH 4.8 than at pH 6.5 (ASW controls) on filaments, while the reverse tendency was observed on lamellae (Fig. 1). On days 1 and 22, significantly lower counts of mucous cells were observed on lamellae compared with control fish. No significant changes in the number of chloride cells followed the pH 4.8 exposure (Fig. 2).

Interlamellar distance increased significantly following 1 d of exposure to pH 4.8 but was not significantly different from controls throughout the remainder of exposure (Table 1). The thickest filaments were found on days 3, 8, and 81, in association with generally thicker filamental epithelia at these times (Table 1). Significant treatment effects (ANOVA) were also found for thickness of both secondary lamellae and lamellar epithelium, but these changes were too small to be detected by a posteriori tests (Table 1). The net effect of these small variations, however, was reflected in the water–blood diffusion distance measurements, with a tendency for reduced diffusion distance throughout the period of acid exposure (Table 1). This narrowing of diffusion distance was most pronounced on day 22 when a significant 25% decrease was observed compared with the diffusion distance in ASW control and day 3 fish.

Light microscopy observations revealed that apical hyperplasia (Fig. 3a) and club deformations (Fig. 3b) were frequent features in ASW control fish as well as in pH 4.8 exposed fish. Most of the lamellar mucous cells were concentrated at the tip of lamellae (Fig. 3c). In contrast, lamellar chloride cells were found all along the lamellae in controls and in fish exposed to pH 4.8 for 1, 3, and 8 d (Fig. 3d and 3e). However, they were more concentrated at the base of lamellae after 22 and 81 d of sublethal acid exposure (Fig. 3f and 3g). Additional qualitative observations of chloride cells in control and day 81 fish were carried out by transmission electron microscopy. No marked differences were present between chloride cells of the two groups. However, apoptosis (cf. Wendelaar Bonga and van der Meij 1989) was more frequent in ASW control fish, and the presence of rough endoplasmic reticulum was more prominent in long-term acid-exposed fish than in controls.

No significant changes in total gill ATPase activity or in Na^+–K^+-ATPase activity were observed during this experiment. The overall average values (controls and long-term acid-stressed fish) obtained were, for total gill activity, 368 ± 14.8 (50) μmol PO_4·g gill tissue^{-1}·h^{-1} and 4.9 ± 0.29 (48) μmol PO_4·mg protein^{-1}·h^{-1}, and for Na^+–K^+-ATPase activity, 61 ± 4.1 (51) μmol PO_4·g gill tissue^{-1}·h^{-1} and 0.8 ± 0.05 (49) μmol PO_4·mg protein^{-1}·h^{-1}.

Following transfer to pH 4.8, plasma cortisol increased to a maximal level on day 3 and stayed high until the end of the experiment (Table 2). Adrenaline and noradrenaline, although highly variable, also reached a maximum concentration on day 3 (Table 2). On other days, plasma concentrations were not significantly elevated, and by day 81, noradrenaline levels were equal to and adrenaline levels were significantly below those of control fish.

Acute Severe Acid Challenge

Changes in identifiable mucous and chloride cell numbers were the most prominent effects of pH 4.0 challenge on gill morphology. In naive fish, a significant increase in the number of visible mucous cells on both filaments and lamellae followed pH 4.0 challenge for 5 h (Fig. 4). In fish preexposed to pH 4.8 for 3 mo, the rise was only significant on lamellae. However, when the two pH 4.0 challenge groups were compared, no significant difference was found between them. Both naive and chronically acid-preexposed fish showed a significant decrease of approximately 35% in the number of chloride cells on filaments following 5 h of challenge at pH 4.0 (Fig. 4). Many chloride cells appeared necrotic and in the process of lifting out of the epithelia. Final numbers of chloride cells on filaments

TABLE 1. Effect of long-term sublethal exposure on gill morphometry. Results are presented as mean ± SEM (n), n indicating the number of fish. Results of the a posteriori tests of comparison of means are indicated by superscript letters a and b. Means with the same superscript letter are not significantly different from one another. Asterisks represent significant differences from controls (C).

Day	Interlamellar distance (μm)	Thickness of filaments (μm)	Thickness of filamental epithelium (μm)	Thickness of secondary lamellae (μm)	Thickness of lamellar epithelium (μm)	Diffusion distance (μm)
C (pH 6.5)	21.6 ± 0.95^a (6)	88.9 ± 7.97^a (6)	28.0 ± 2.26^{ab} (6)	14.9 ± 0.67^a (6)	4.1 ± 0.28^a (6)	5.6 ± 0.30^b (6)
1	27.3 ± 1.87^{b*} (6)	85.1 ± 11.75^a (6)	21.8 ± 3.06^a (6)	12.6 ± 0.90^a (6)	3.4 ± 0.40^a (5)	4.4 ± 0.39^{ab} (5)
3	21.9 ± 1.01^{ab} (7)	157.9 ± 16.98^{b*} (7)	40.9 ± 4.01^b (7)	15.1 ± 0.63^a (7)	3.9 ± 0.23^a (7)	5.4 ± 0.30^b (7)
8	23.5 ± 1.12^{ab} (7)	119.8 ± 16.6^{ab} (7)	34.4 ± 3.33^{ab} (7)	14.1 ± 0.64^a (7)	4.1 ± 0.30^a (7)	4.8 ± 0.26^{ab} (7)
22	20.3 ± 0.95^a (7)	71.06 ± 7.64^a (7)	22.7 ± 2.95^a (7)	12.5 ± 0.45^a (7)	3.0 ± 0.20^a (7)	4.2 ± 0.23^{a*} (7)
81	22.4 ± 1.39^{ab} (7)	118.6 ± 11.91^{ab} (7)	28.1 ± 2.79^{ab} (7)	15.0 ± 0.62^a (7)	3.8 ± 0.24^a (7)	5.1 ± 0.23^{ab} (7)

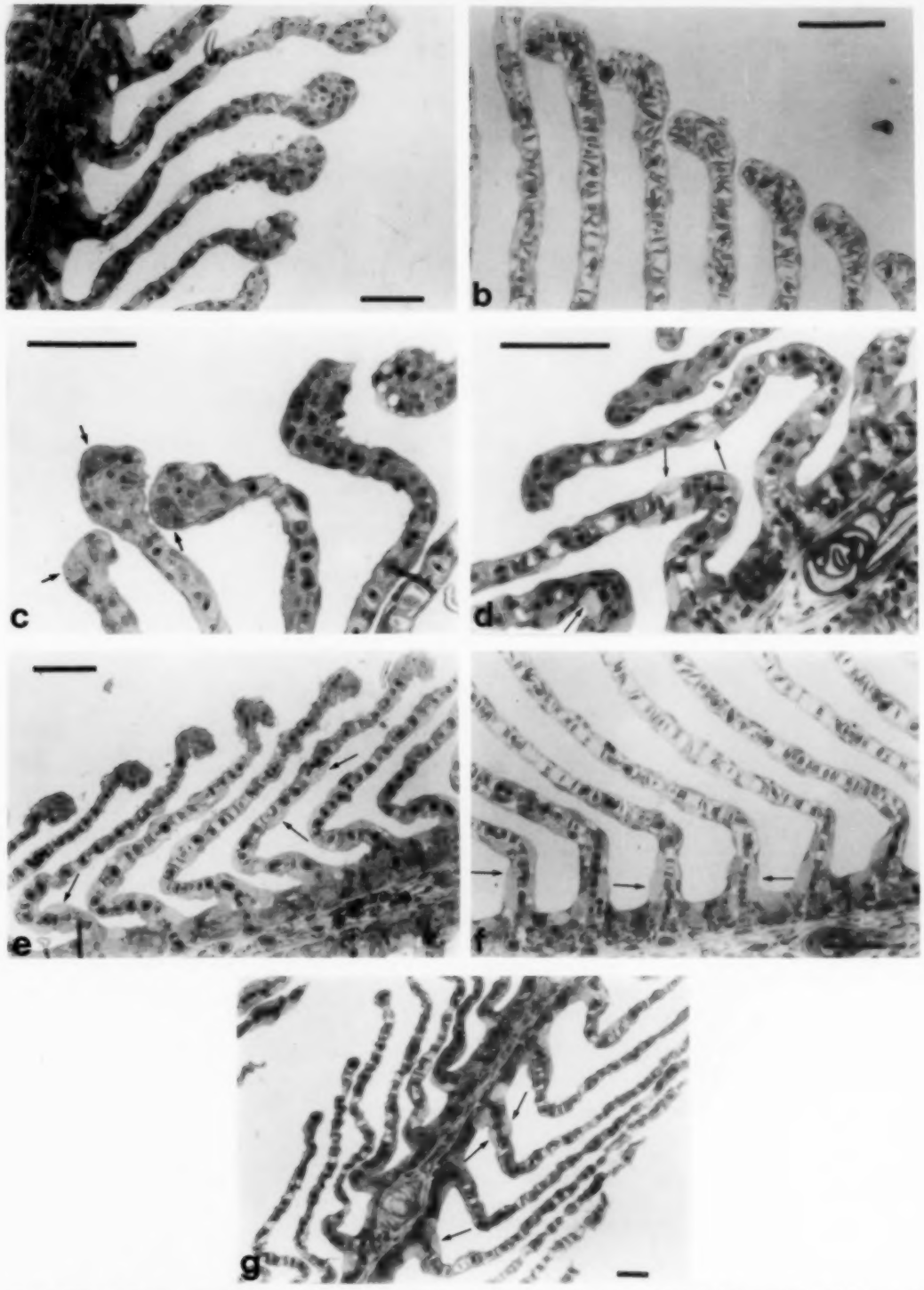

FIG. 3. Effects of long-term sublethal acid exposure on gill morphology in rainbow trout. (a) Apical hyperplasia, ASW control; (b) club deformation, pH 4.8, day 81; (c) mucous cells on lamellar tips, ASW control; (d) lamellar chloride cells in ASW control; (e) lamellar chloride cells, pH 4.8, day 3; (f) lamellar chloride cells, pH 4.8, day 81; (g) lamellar chloride cells, pH 4.8, day 22. Short arrows: mucous cells; long arrows: chloride cells; scale bars = 50 μm.

TABLE 2. Effect of long-term sublethal acid exposure on plasma cortisol and catecholamines. Results are presented as mean ± SEM (*n*), *n* indicating the number of fish. Results of the a posteriori tests of comparisons of means are indicated by superscript letters a to e. Means with the same superscript letter are *not* significantly different from one another. Asterisks represent significant differences from controls (C).

Day	Cortisol ($ng \cdot mL^{-1}$)	Adrenaline ($nmol \cdot L^{-1}$)	Noradrenaline ($nmol \cdot L^{-1}$)
C (pH 6.5)	42.6 ± 5.5^{ab} (23)	19.8 ± 3.67^{bcd} (20)	9.0 ± 1.54^{bcd} (20)
1	52.9 ± 17.2^{abc} (17)	8.7 ± 2.66^{ab} (7)	12.9 ± 1.83^{a*} (7)
3	128.1 ± 25.4^{d*} (16)	57.5 ± 24.50^{e*} (16)	22.8 ± 8.30^{ab} (16)
8	84.3 ± 17.9^{cd*} (7)	12.4 ± 3.80^{ab} (7)	6.3 ± 1.61^{cde} (7)
22	65.9 ± 2.2^{bcd} (5)	12.3 ± 2.64^{abc} (5)	3.2 ± 0.26^{e} (5)
50	121.8 ± 37.0^{d*} (7)	41.1 ± 19.95^{ce} (7)	8.7 ± 2.02^{bcde} (7)
81	96.1 ± 26.0^{cd*} (7)	9.4 ± 4.59^{a*} (7)	7.7 ± 3.73^{de} (7)

were similar between the two groups challenged with pH 4.0. Acute pH 4.0 challenge also produced a decrease of almost 50% in chloride cell numbers on the secondary lamellae of naive fish. This effect was not seen upon pH 4.0 challenge in the acid-preexposed fish, but this group already exhibited somewhat lower chloride cell numbers on lamellae. As with filamental chloride cells, no significant difference was found on lamellar chloride cell numbers between the two groups of fish challenged with pH 4.0 (Fig. 4).

A significant 42% increase in interlamellar distance was observed in naive fish challenged with pH 4.0 (Table 3). The response was similar to that seen after exposure of naive fish to pH 4.8 for 1 d (Table 1). This effect did not occur when acid-preexposed fish were challenged with pH 4.0. The difference between the two pH 4.0 groups was significant. The 5-h challenge at pH 4.0 did not induce any changes in thickness of the filaments or secondary lamellae, thickness of their epithelia, or water–blood diffusion distance in either naive or acid-preexposed fish (Table 3).

Light microscopy demonstrated the presence of apical hyperplasia in both pH 4.0 challenged groups, similar to that in the naive and long-term acid-preexposed fish. The lamellar epithelium was often damaged and separated in fish challenged with pH 4.0 (Fig. 5a and 5b). Severe lamellar swelling was observed in two of the naive fish challenged with pH 4.0 (Fig. 5c). This damage to the lamellar epithelium may have impaired cell type identification, leading to artificially reduced cell numbers. Nevertheless, as noted above, lamellar mucous cell numbers were significantly increased in this group. This increase was caused by an increased number of visible mucous cells along the lamellae (Fig. 5d) instead of only at the tips as seen at pH 4.8.

Again, no significant differences in total gill ATPase activity or gill Na^+–K^+-ATPase activity resulted from severe acid exposure in either treatment group. Values were, for total gill activity, 342 ± 22.0 (28) μmol $PO_4 \cdot g$ gill $tissue^{-1} \cdot h^{-1}$ and 4.8 ± 0.47 (28) μmol $PO_4 \cdot mg$ $protein^{-1} \cdot h^{-1}$, and for Na^+–K^+-ATPase activity, 59 ± 4.0 (28) μmol $PO_4 \cdot g$ gill $tissue^{-1} \cdot h^{-1}$ and 0.8 ± 0.08 (28) μmol $PO_4 \cdot mg$ $protein^{-1} \cdot h^{-1}$.

Naive fish exposed to pH 4.0 exhibited a threefold increase in their plasma cortisol concentration (Fig. 6). In acid-preexposed fish, the transfer to pH 4.0 did not significantly increase plasma cortisol concentration, but levels were already very high in these fish. There was no significant difference in plasma cortisol between the two pH 4.0 challenged groups. No significant differences were observed in plasma concentrations of adrenaline and noradrenaline following the pH 4.0 exposure in either naive or acid-preexposed fish; average plasma adrenaline concentration was 25.7 ± 8.39 (5) $nmol \cdot L^{-1}$ in challenged naive fish and 18.6 ± 7.2 (5) $nmol \cdot L^{-1}$ in challenged acid-preexposed fish, while plasma noradrenaline concentration was 17.6 ± 6.17 (5) and 11.7 ± 2.28 (5) $nmol \cdot L^{-1}$ in the two challenged groups, respectively.

Discussion

The most important finding of the present study is that only minor changes in gill morphology occurred during long-term sublethal acid stress. These results are consistent with some previous field and laboratory studies on salmonids. In particular, Lacroix et al. (1990) found more or less unchanged gill features in feral brook trout (*Salvelinus fontinalis*) and juvenile Atlantic salmon (*Salmo salar*) exposed naturally to a range of different pH and Al concentrations in softwater streams of Nova Scotia. In that study, the high organic carbon concentrations of the water apparently protected against Al toxicity, and sublethal physiological effects similar to those observed by Audet et al. (1988) were attributed to acidity alone. Karlsson-Norrgren et al. (1986) reached a similar conclusion with respect to the lack of branchial morphological damage accompanying extended exposure of brown trout (*Salmo trutta*) to pH 5.5 plus Al in humate-enriched softwater. The studies of Jagoe and Haines (1983), Evans et al. (1988), and Mueller et al. (1991) on Sunapee (*Salvelinus alpinus aquassa*), rainbow trout, and brook trout, respectively, also indicated minimal changes in gill morphology in fish exposed to pure sublethal acid stress.

In contrast, a number of other studies on both salmonid (Daye and Garside 1976; Tietge et al. 1988; Brown et al. 1990a; Jagoe and Haines 1990; Laurent and Perry 1991) and nonsalmonid species (Leino and McCormick 1984; Leino et al. 1987; Wendelaar Bonga et al. 1990) have reached a different conclusion, even though acidity alone was thought to be the major or sole stressor. These investigations have documented a variety of pronounced structural changes in the gills (chloride cell proliferation and/or degeneration, mucous cell proliferation, hyperplasia of the lamellar and/or filamental epithelia, increases in the blood–water diffusion distance). Of these, only slight increases in filamental mucous and chloride cell numbers and a variable thickening of the filamental epithelium were seen in the present investigation, and the blood–water diffusion distance in the lamellae actually declined. The reasons for this disagreement are unclear but may include differences in the severity of acid exposure, water chemistry (especially hardness), species, and the involvement of metals or other toxic agents in those studies showing pronounced effects.

The present results certainly support the hypothesis of McDonald and Wood (1992) that at the branchial level, significant structural damage is a necessary prerequisite of acclimation and that acclimation is associated with damage repair. Furthermore, they agree with the majority of the literature (reviewed by Audet et al. 1988; Audet and Wood 1988; Wood 1989) which indicates that acclimation to acidity alone does not occur or is at best equivocal. However, this does not deny the

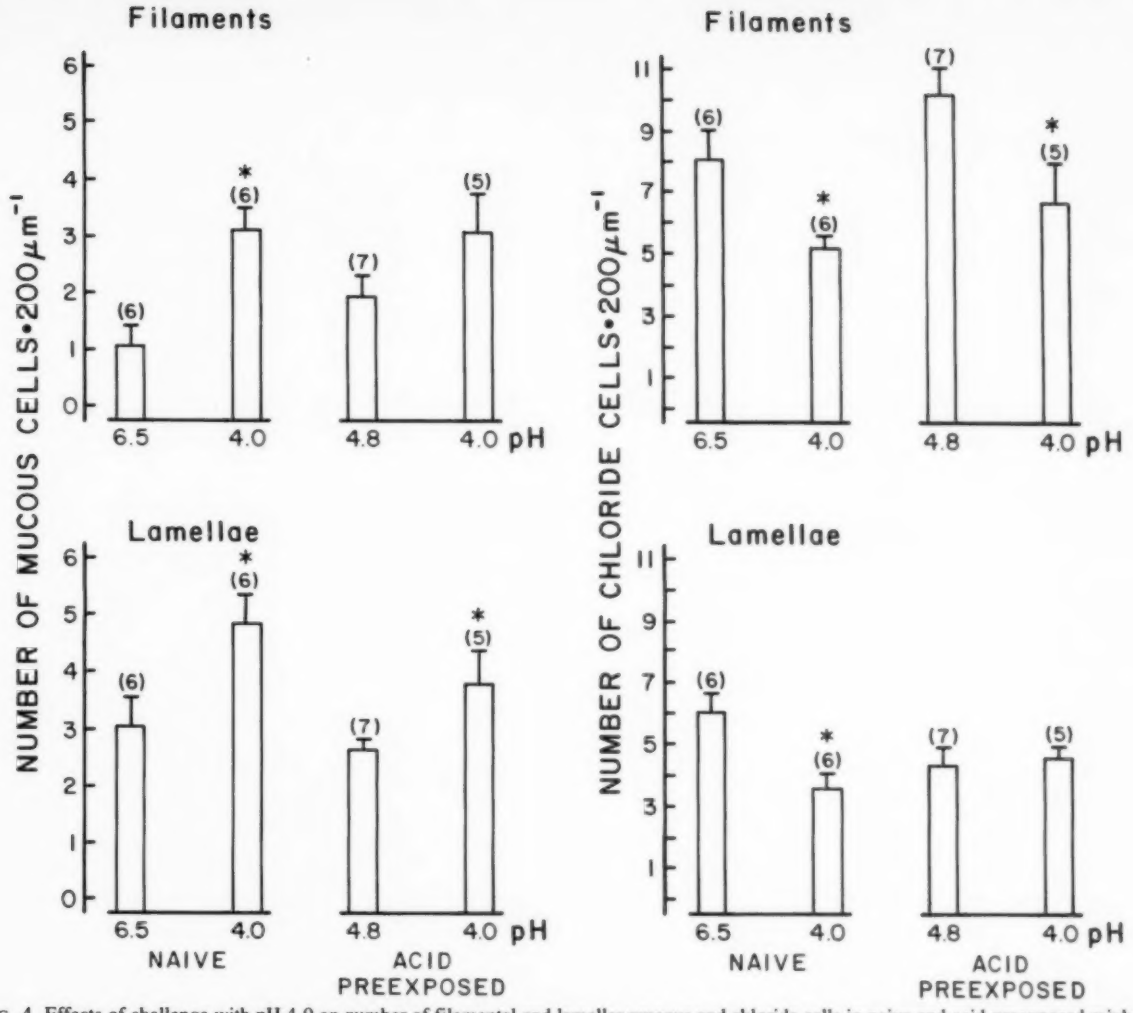

FIG. 4. Effects of challenge with pH 4.0 on number of filamental and lamellar mucous and chloride cells in naive and acid-preexposed rainbow trout. Asterisks represent significant effects ($p \leq 0.05$) of pH 4.0 challenge in naive fish (column 1 versus column 2) or in acid-preexposed fish (column 3 versus column 4). Numbers of fish in parentheses.

possibility of acclimation to acidity, given the right set of circumstances. Indeed, we would predict that acclimation should have occurred in those studies listed above where significant damage repair was seen during exposure. However, as challenge tests were not performed in any of these studies, this remains hypothetical. Nevertheless, it is noteworthy that the tilapia (*Oreochromis mossambicus*), which is much more resistant to low pH than the rainbow trout, exhibited substantial initial gill damage followed by pronounced repair, most prominently a proliferation of chloride cells, during exposure to pH 4.5 (Wendelaar Bonga et al. 1990). At the same time, complete restoration of plasma electrolytes occurred (Wendelaar Bonga et al. 1984). Such complete recovery in the continued presence of the stressor is often indicative of acclimation (McDonald and Wood 1992).

In the present study, there was no recovery of plasma Na^+ and Cl^- during chronic exposure to pH 4.8, but rather an eventual stabilization at a reduced level. There was, however, a partial restoration of the initially inhibited J_{in} components, while the initially inhibited J_{out} components remained low, such that the J_{net} values for both ions returned to control levels by day 81. By analogy to other studies (Leino and McCormick 1984; Avella et al. 1987), we originally hypothesized that the partial J_{in} recovery was the result of an increased number of transport sites via an increase in chloride cell numbers (Audet et al. 1988). The current observations show this hypothesis to be false. No significant changes in chloride cell numbers were observed on either filaments or lamellae, although their distribution on the lamellae was altered. There was a tendency for concentration of chloride cells at the base of the lamellae after chronic sublethal exposure.

Control animals at pH 6.5 already exhibited numerous chloride cells on lamellae, probably resulting from the softwater acclimation employed to duplicate realistic field conditions. Various authors (Laurent et al. 1985; Perry and Wood 1985; Avella et al. 1987; Spry and Wood 1988; Laurent and Hebibi 1989; Perry and Laurent 1989; Laurent and Perry 1991) have proposed that the high number of chloride cells found on lamellae of trout exposed to soft water is an adaptive response to enhance active ion influx in a dilute environment. In the present

TABLE 3. Comparison of gill morphology before and after a 5-h challenge at pH 4.0 between fish never exposed to sublethal acid stress (naive, pH 6.5) and fish preexposed for 81 d to pH 4.8.

Gill parameter	Naive fish		Acid-preexposed fish	
	Before	After	Before	After
Interlamellar distance (μm)	21.6 ± 0.95 (6)	27.6 ± 2.10[a] (6)	22.4 ± 1.39 (6)	19.1 ± 1.37[b] (5)
Thickness of filaments (μm)	88.9 ± 7.97 (6)	96.5 ± 8.39 (6)	118.6 ± 11.89 (7)	119.6 ± 17.12 (5)
Thickness of filamental epithelium (μm)	28.0 ± 2.26 (6)	30.8 ± 1.39 (6)	28.1 ± 2.79 (7)	28.2 ± 3.51 (5)
Thickness of secondary lamellae (μm)	14.9 ± 0.67 (6)	15.2 ± 0.79 (6)	15.0 ± 0.62 (7)	15.3 ± 0.61 (5)
Thickness of lamellar epithelium (μm)	4.1 ± 0.27 (6)	4.0 ± 0.17 (6)	3.8 ± 0.24 (7)	3.6 ± 0.21 (5)
Diffusion distance (μm)	5.6 ± 0.30 (6)	5.6 ± 0.23 (6)	5.1 ± 0.23 (7)	5.2 ± 0.29 (5)

[a]Significant effect ($p \leq 0.05$) of pH 4.0 challenge in naive fish.
[b]Significant difference between naive and acid-preexposed fish following challenge with pH 4.0.

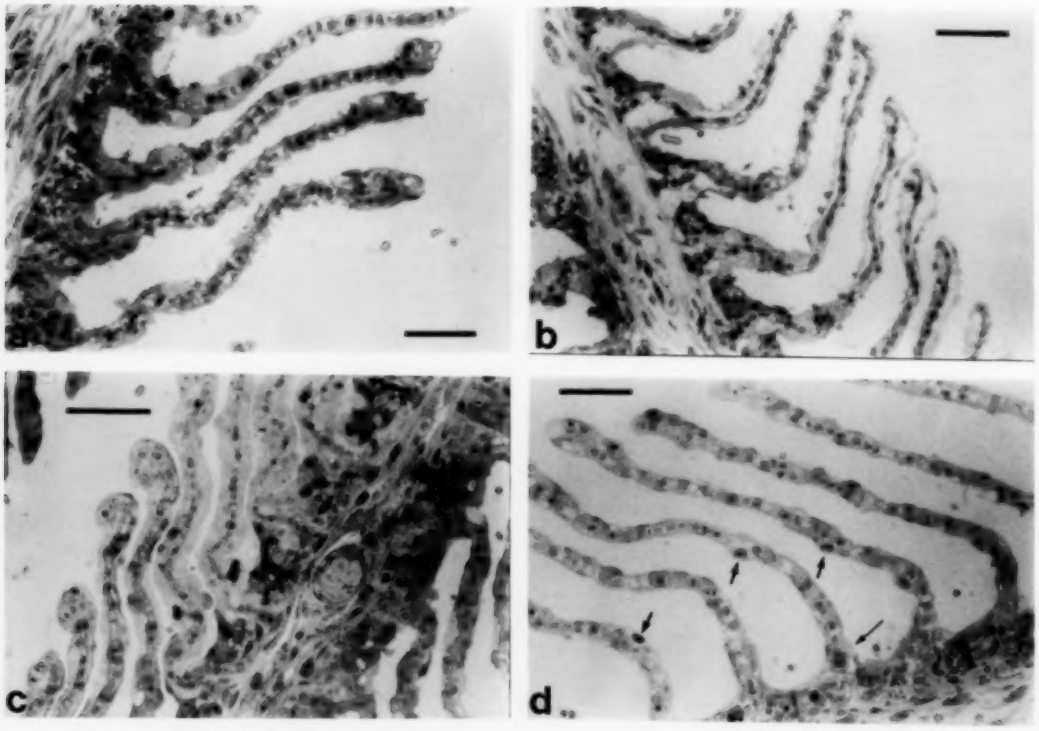

FIG. 5. Effects of acute pH 4.0 challenge on gill morphology in rainbow trout. (a and b) Damage to the lamellar epithelium following pH 4.0 challenge in acid-preexposed fish; (c) swelling of the lamellar epithelium following pH 4.0 challenge in naive fish; (d) lamellar mucous cells following pH 4.0 challenge in naive fish. Short arrows: mucous cells; long arrows: chloride cells; scale bars = 50 μm.

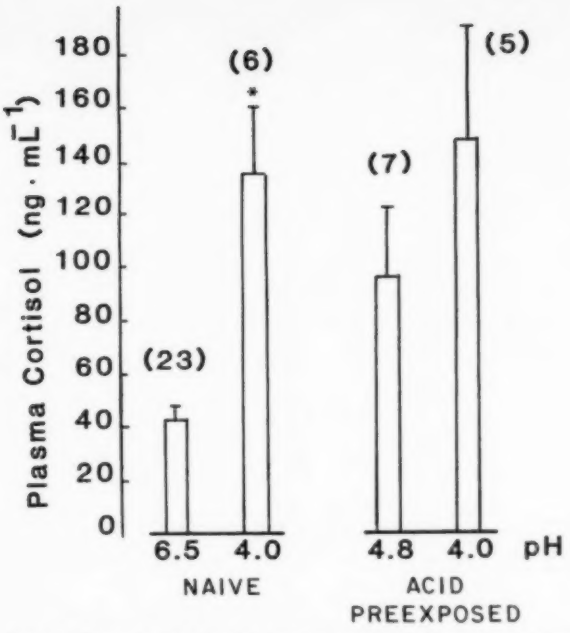

FIG. 6. Effects of pH 4.0 challenge on plasma cortisol concentration in naive and acid-preexposed rainbow trout. Asterisk represents a significant effect ($p \leq 0.05$) of pH 4.0 exposure in naive fish (column 1 versus column 2). Numbers of fish in parentheses.

experiment, we speculate that the softwater-acclimated trout had already fully recruited the potential offered by chloride cell proliferation to increase ion uptake.

The question then arises: what mechanism(s) could explain the partial recovery of influx observed in chronically exposed rainbow trout? There was no evidence of increased enzymatic activity of chloride cells following long-term sublethal acid exposure, at least as indicated by Na^+–K^+-ATPase activity. A decrease in Na^+–K^+-ATPase and/or total ATPase activity in the gills has been observed in several other studies on acid-exposed salmonids (Nieminen et al. 1982, pH 5.0; Saunders et al. 1983, pH 4.2–4.7; Staurnes et al. 1984, pH 5.0). Electron microscopy also provided no definitive evidence for an increase in chloride cell activity in the present study. Apical pits appeared to be absent from the chloride cells in both groups, in sharp contrast with the observations of Leino and McCormick (1984), Leino et al. (1987), and Wendelaar Bonga et al. (1990) on fathead minnow (*Pimephales promelas*) and tilapia chronically exposed to sublethal low pH. However, there was one subtle difference in ultrastructure between the chloride cells of control and 3-mo acid-exposed trout. Apoptosis (degeneration preceding physiologically controlled cell death; cf. Wendelaar Bonga and van der Meij 1989) seemed to be more frequent in control fish, and prominent rough endoplasmic reticulum more frequent in experimental fish. This may correlate with the slightly greater chloride cell numbers observed on the filaments and at the base of the lamellae in long-term acid-exposed trout. Cells in this position are thought to be younger (Conte and Lin 1967; Zenker et al. 1987). Therefore the actual number of "healthy," fully functioning chloride cells may have been greater in long-term acid-exposed fish. Whether this factor alone was responsible for the partial recovery of J_{in} values remains unresolved.

In the present study, the fish reduced $J_{out}^{Na^+}$ and $J_{out}^{Cl^-}$ immediately upon exposure to pH 4.8 and maintained this reduction throughout the 3-mo experiment (Audet et al. 1988). We originally hypothesized that this response to sublethal pH could be the consequence of a decrease of membrane permeability through endocrine modulation and/or the inhibition of the exchange diffusion component (Na^+/Na^+ and Cl^-/Cl^- exchange) at the gills. In the model presented by McDonald and Prior (1988) on branchial exchanges in rainbow trout, simple passive effluxes (not involving exchange diffusion) would be achieved largely through tight junctions on paracellular channels. This aspect of membrane permeability was not surveyed by the present study. However, thickness of the filaments did tend to increase under chronic acid exposure, although the response was not immediate. Thickening probably resulted from the proliferation of undifferentiated epithelial cells, as chloride cell numbers did not change and alterations in mucous cell numbers did not parallel the changes in filamental thickness. Filamental thickening has been documented in other salmonid species exposed to low pH with or without parallel Al contamination (Chevalier et al. 1985; Evans et al. 1988; Brown et al. 1990a; Jagoe and Haines 1990). Brown et al. (1990a) interpreted the response as a way to reduce ion permeability. However, in the present study, this would likely have only a small impact considering the much larger exchange surface offered by lamellae and the fact that diffusion distance on lamellae actually decreased under chronic acid exposure. We conclude that inhibition of exchange diffusion, which would likely have no morphological correlate, was probably the more important contributor to the immediate and sustained reductions in J_{out} values.

The decrease in blood–water diffusion distance in the lamellae during chronic acid exposure was surprising. Such a decrease would tend to facilitate instead of limit passive ion fluxes. However, it is noteworthy that Laurent and Hebibi (1989) observed the same phenomenon after transfer of rainbow trout to ion-poor water. These investigators suggested that the response served to facilitate the transcellular diffusive efflux of NH_3 at a time when Na^+/NH_4^+ exchange was likely inhibited. Low environmental pH is also thought to inhibit Na^+/NH_4^+ exchange (Wright and Wood 1985). The present fish exhibited a sustained increase in NH_3 efflux, apparently a diffusive NH_3 efflux, throughout acid exposure (Audet et al. 1988).

Increased numbers of mucous cells on filaments and lower numbers on lamellae were observed during chronic exposure to pH 4.8. Mucous cell hyperplasia and hypertrophy on filaments have been reported previously in acid-stressed salmonids (Daye and Garside 1976; Jagoe and Haines 1990; Laurent and Perry 1991). McDonald (1983) suggested that increased mucous production during acidification may decrease the loss of ions by passive diffusion.

The major physiological and minor morphological adjustments which occurred under chronic sublethal acid exposure did not confer increased tolerance to acute acid challenge (pH 4.0). Indeed, increases in both $J_{out}^{Na^+}$ and $J_{out}^{Cl^-}$ were almost twice as large as in naive fish (Audet and Wood 1988). In general, the morphological changes seen after 5 h of acute acid exposure in both groups were similar and in accord with those observed in many previous studies employing severe acid challenge (e.g. Daye and Garside 1976; Jagoe and Haines 1983; Mallatt 1985; McDonald et al. 1991). Damage was not as severe as in some of these studies. Nevertheless, the general epithelial damage, the loss of identifiable chloride cells by necrosis, and the increase in visible mucous cells were the expected

responses. Chloride cells were clearly the most damaged cell type, many being lifted out from the epithelia and difficult to identify under the light microscope. This lifting may be the cause of the increased interlamellar distance observed in naive fish after a 5-h challenge at pH 4. However, increased interlamellar distance was also recorded after 1 d of exposure to pH 4.8 without simultaneous observation of chloride cell necrosis or lifting. Furthermore, interlamellar distance did not increase when acid-preexposed fish were challenged with pH 4.0, even though these fish had a greater concentration of chloride cells at the base of the lamellae. These observations suggest that an additional mechanism must be involved.

Plasma cortisol is considered a good primary stress indicator and plasma glucose a good secondary stress indicator in fish (Donaldson 1981; Wedemeyer and McLeay 1981). In long-term acid-exposed fish, the plasma cortisol concentration rose and stayed elevated for the whole duration of the experiment, in accord with the prolonged elevation in blood glucose (Audet et al. 1988). These observations indicate that the fish remained under a high level of stress throughout the exposure and are in accord with the lack of acclimation. Previous investigations have produced conflicting results about the cortisol response to chronic sublethal acid stress. Most studies have shown a transient rise in cortisol levels (reviewed by Wendelaar Bonga and Balm 1989), while others have shown that the increases were of longer duration (Brown et al. 1984, 1986a, 1986b, 1990b; Tam et al. 1987, 1988; Whitehead and Brown 1989). Tam et al. (1988) also showed that acid exposure results in hypertrophy and activation of corticotropes and interrenal cells. In the present study, the sustained increase in cortisol probably contributed to both the sustained elevation in NH_3 excretion and the hyperglycaemia observed during chronic sublethal acid exposure (Audet et al. 1988). Cortisol is also reported to cause chloride cell proliferation on the gills, thereby increasing ion uptake (Perry and Wood 1985; Laurent and Perry 1990). The present morphological results do not concur; however, it is possible that elevated cortisol levels were important in maintaining chloride cell numbers more or less unchanged and in promoting their transport function in the face of potential inhibition by acidity.

Catecholamines are generally agreed to raise blood glucose (Mazeaud and Mazeaud 1981) and have an as yet controversial role in the control of branchial ion transport (Wood 1991). Concentrations increased only transiently (day 3) during chronic exposure in contrast with the sustained changes in plasma cortisol, glucose, ions, and branchial Na^+ and Cl^- fluxes. Similarly, catecholamines exhibited little response to acute pH 4.0 challenge, in contrast with the other parameters. While this suggests that catecholamines were relatively unimportant in the observed phenomena, it must be noted that control levels were high and the data variable (cf. Witters et al. 1991) in the present study. Catecholamine release is known to occur extremely rapidly when fish are disturbed (Mazeaud and Mazeaud 1981); the present data were probably confounded by the stress of capture, anaesthesia, and caudal sampling such that small variations due to acid exposure would have been undetectable. However, Witters et al. (1991), using a cannulation approach to avoid sampling disturbance, saw no change in plasma adrenaline or noradrenaline when rainbow trout were exposed to pH 5.0 for 2 d. Similarly, Ye et al. (1991) found that catecholamine levels did not change in rainbow trout exposed to pH 4.0 (for up to 72 h) until immediately before death, when they increased abruptly. There is clearly a need for longer term studies on this question employing sampling by cannulation.

Acknowledgements

Supported by an NSERC Strategic Grant in Environmental Quality to C.M.W. We thank Linda Allen, Evie Pertens, Steve Munger, and Carolina Migoya for excellent technical assistance.

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Genetic and Morphological Differentiation between Dwarf and Normal Size Forms of Lake Whitefish (*Coregonus clupeaformis*) in Como Lake, Ontario[1]

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Normal and dwarf size forms of lake whitefish (*Coregonus clupeaformis*) from Como Lake, Ontario, were sampled at spawning time and examined for differences in electrophoretic, mtDNA, and morphological characteristics to test the hypothesis of reproductive isolation and provide clues regarding evolutionary origin. Of the 36 enzyme loci examined, 33 were fixed for the same alleles in both dwarf and normal lake whitefish. At the three polymorphic loci, allele frequencies were not statistically different between dwarfs and normals. mtDNA analysis revealed five different haplotypes. The same mtDNA haplotype was the most common in both dwarf and normal lake whitefish, but there was a statistically significant difference in haplotype frequencies between the two size forms. Discriminant and principal component analyses demonstrated highly significant morphological differences between dwarfs and normals. Because the two size morphs spawn in the same place at the same time, it is most likely that genetic differences, not different rearing environments, underly the observed morphological differences. mtDNA haplotypes derived from both the Mississippi and Atlantic glacial refugia are present in Como Lake lake whitefish, raising the possibility of an allopatric divergence for the two forms; however, this hypothesis requires testing by genetic comparisons of other sympatric populations in Ontario.

On a échantillonné les formes normale et naine du grand corégone (*Coregonus clupeaformis*) du lac Como (Ontario) au moment de la fraye, et l'on a examiné les différences de migration électrophorétique, l'ADNmt et les caractéristiques morphologiques, afin de vérifier l'hypothèse relative à l'isolement reproductif et d'obtenir des indices concernant leur origine évolutive. Sur les 36 loci enzymatiques étudiés, 33 présentaient les mêmes allèles chez les formes naine et normale du grand corégone. Dans le cas des trois loci polymorphes, on n'a pas observé de différence statistique entre les fréquences des allèles chez les deux formes. L'analyse de l'ADNmt a révélé la présence de cinq haplotypes différents. On a constaté que le même haplotype d'ADNmt était le plus commun à la fois chez les grands corégones nains et ceux de taille normale, mais qu'il y avait une différence statistique importante dans la fréquence des allèles chez les deux formes. Les analyses discriminantes et les analyses en composantes principales indiquent qu'il existe des différences morphologiques très importantes entre les formes naine et normale. Ces formes de taille différente frayant toutes deux au même endroit et au même moment, il est fort probable que les différences morphologiques observées soient dues à des différences génétiques plutôt qu'à des différences dans les milieux où se produit l'alevinage. Les haplotypes d'ADNmt provenant du refuge glaciaire naturel du Mississippi et de celui de l'Atlantique sont présents chez le grand corégone du lac Como, ce qui indique la possibilité d'une divergence de nature allopatrique chez les deux formes; toutefois, cette hypothèse doit être vérifiée par des comparaisons génétiques avec d'autres populations sympatriques en Ontario.

Received December 3, 1991
Accepted July 8, 1992
(JB317)

Reçu le 3 decembre 1991
Accepté le 8 juillet 1992

Many salmonid species include sympatric populations, which are often characterized by distinct life histories and can be separated by their morphology and/or genetic properties (Behnke 1972). The origin and isolating mechanisms of these sympatric populations are of particular interest in the study of speciation mechanisms. The two contrasting views of speciation for such sympatric populations are that they have either developed in sympatry or are the result of secondary contact between two forms which diverged allopatrically.

The lake whitefish (*Coregonus clupeaformis*) in North America constitutes a species complex with five glacial races (Bernatchez and Dodson 1991; Bodaly et al. 1992; Foote et al. 1992). The races have distinct genetic characters and their distribution is mainly allopatric. In addition, several instances of sympatric lake whitefish forms within the same lake are known (Lindsey et al. 1970; Bodaly 1979; Bernatchez and Dodson 1990a). Most of these forms are easily recognizable by differ-

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ences in their size and gill raker count. The presence of an additional sympatric pair of lake whitefish was recently documented in Como Lake (Bodaly et al. 1991a). The size distribution of spawning fish was distinctly bimodal with modes at 170–179 and 280–289 mm fork length. For both forms, the modal gill raker count was 24, but dwarfs had significantly lower mean gill raker counts (24.4 versus 25.0). Besides size, dwarfs and normals also differed significantly in growth rate, reproductive age, and generation length. Dwarfs grew much slower, matured earlier, and their generation length was short compared with normals (Bodaly et al. 1991a). Thus, dwarfs compensate their lower fecundity by earlier maturity and shorter generation length. The objective of this study was to investigate genetic (isozyme and mtDNA) and morphological differences between the sympatric lake whitefish populations in Como Lake in order to (1) test the hypothesis that the sympatric populations are reproductively isolated and (2) provide clues regarding the evolutionary origin of the sympatric pairs.

Materials and Methods

Como Lake is located in northern Ontario (47°55′N, 83°30′W), near the town of Chapleau, in the Michipicoten River basin tributary to Lake Superior. It has a surface area of 1596 ha, a mean depth of 9.4 m, and a typical coolwater fish community, with the exception that no ciscoes are present (Bodaly et al. 1991a).

Lake whitefish were captured with gill nets of mesh sizes 3.8–13.3 cm stretched measure on a spawning shoal overnight on 25–26 October 1989. Additional samples for mtDNA analyses were captured on 28–30 August 1990 with similar gill nets, away from spawning areas. Fish were immediately placed on ice and frozen within 12 h of capture. Eggs were stripped from 44 normals and 36 dwarfs and transported on ice to the laboratory for mtDNA extraction.

The isozyme products of 36 genetic loci were analyzed by starch gel electrophoresis as described in Bodaly et al. (1991b). The enzyme loci scored were the same as given in Bodaly et al. (1991b) except for the omission of *CK-B* and *LDH-C*, expressed in eye tissue, and the addition of esterase (*EST*) which is most strongly expressed in liver.

mtDNA was purified from liver and egg tissues by a rapid extraction method (Bernatchez et al. 1988). mtDNA aliquots were digested with eight hexameric (*Dra*I, *Hind*III, *Pvu*II, *Sma*I, *Xmn*I, *Bgl*I,*Pst*I, *Bam*HI), four multihexameric (*Ava*I, *Ban*I, *Hae*II, *Hinc*II), and one multipentameric (*Ava*II) restriction enzymes. mtDNA fragments were electrophoretically separated on 0.8 and 1.2% agarose gels at 25 V run overnight. Restriction fragments were revealed by either ethidium bromide staining or hybridization with a highly purified radiolabelled total mtDNA probe (Bernatchez and Dodson 1990b). Fragments were sized by comparison with digests of phage lambda DNA with *Hind*III and *Eco*RI–*Hind*III double digest. No attempt to visualize fragments less than 350 base pairs was made. Distinct single endonuclease patterns were identified by a specific letter in order of appearance. Each fish was assigned a multiletter code which described its composite mtDNA genotype. Intrapopulation diversity of mtDNA lineages was estimated with Nei and Tajima's (1981) nucleon diversity index. Heterogeneity in the frequency of genotypes between populations was analyzed using the likelihood ratio chi-square test (G^2, SAS statistical package).

Nine meristic variables were counted as shown in Fig. 1C and 1E: LLS (lateral line scales), SPS (suprapelvic scales), ULS (scales above the lateral line), DRC (dorsal ray count) (Lindsey 1962), ARC (anal ray count), PEC (pectoral ray count), PVC (pelvic ray count) (Hubbs and Lagler 1974), UGR (upper gill raker count), and LGR (lower gill raker count) (Bodaly 1979).

Twenty morphometric variables were measured (Fig. 1A, 1B, 1D, 1E). The following were measured between topographic features but parallel to the long axis of the body to the nearest 0.1 mm using dial calipers: POL (preorbital length: tip of the snout to the anterior fleshy margin of the orbit), OOL (orbital length: anterior fleshy margin of the orbit to the posterior fleshy margin), PSL (postorbital length: posterior fleshy margin of the orbit to the most posterior projection of the bony operculum), TTL (trunk length: posterior end of the operculum to the origin of the dorsal fin), DOL (dorsal fin length: origin of dorsal fin to the posterior edge of the fin behind the final ray), LUL (lumbar length: end of the dorsal fin to the origin of the anal fin), ANL (anal fin length: origin of anal fin to the posterior edge of the fin), CPL (caudal peduncle length: end of anal fin to the end of the body (structural base of the caudal rays as indicated by a crease when the tail rays are flexed) (Lindsey 1962)), and STL (standard length) (Lindsey 1962). The following variables were measured parallel to the vertical axis of the body: HDD (head depth: vertical depth through the pupil of the eye from the dorsal surface of the cranium to the ventral edge of the gular region), BDD (body depth: vertical depth from the dorsal origin to the ventral surface of the body, and CPD (caudal peduncle depth) (Hubbs and Lagler 1974). IOW (interorbital width), MXL (maxillary length), MXW (maxillary width), and GRL (lower gill raker length) followed Lindsey (1962). The following variables were measured along the axis of the individual body element: PCL (pectoral length: most basal part of the first ray distally to the end of the fin when it is laid flat against the body), PVL (pelvic length: most basal part of the first ray where it forms a crease with the body distally to the most posterior point of the fin when laid flat against the body), ADL (adipose length: distance from the point where skin and scales meet at the anterior end of the fin posteriorly to the free margin of the fin), and LAL (lower arch length) (Bodaly 1979).

Morphometric variables were standardized relative to fish size using both ratios of body part to standard length and analysis of covariance with standard length as the covariate. Residual values, the difference between the observed and predicted size of the body part, from the within-groups regression line for both forms were used to estimate the size of body parts relative to a standard common to both forms (Reist 1985, 1986). The meristic and size-adjusted data sets were analyzed univariately (*t*-tests) and multivariately (discriminant analysis and principal components analysis) to test the hypothesis of identity of the two forms. To ensure that sexual differences did not bias results, all data were initially examined for differences between sexes.

Results

Electrophoresis

Only three of the 36 genetic loci examined were polymorphic, namely *G3PDH-1** and isoloci *sMDH-B1,2**. For both enzyme systems, variation conformed to Hardy–Weinberg expectations. Allele frequencies were almost identical in dwarf and normal lake whitefish at the *G3PDH-1** locus and were not significantly different at isoloci *sMDH-B1,2** (Table 1). In the remaining 33 loci, both size forms were fixed for the same alleles. Both forms

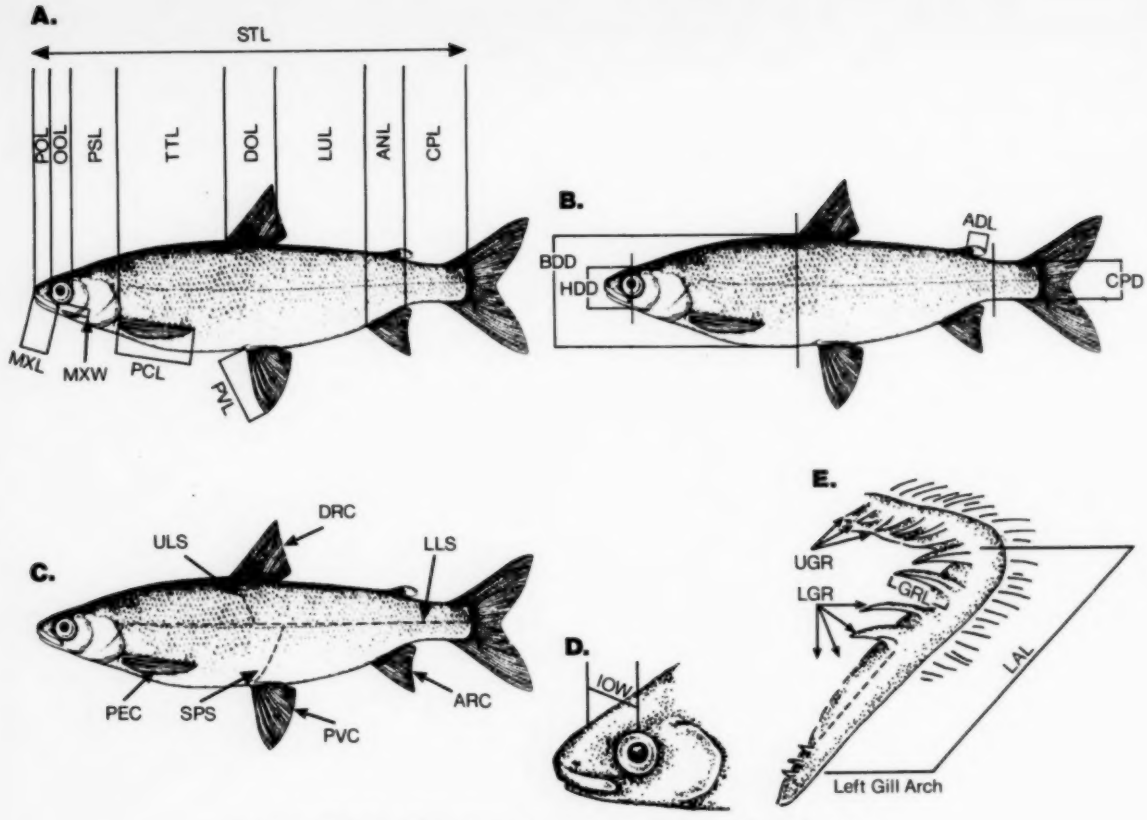

FIG. 1. Meristic and morphometric measurements. See text for abbreviations.

TABLE 1. Allele frequency estimates for dwarf (n = 51) and normal (n = 50) lake whitefish in Como Lake, Ontario. The remaining 33 loci were fixed for the same allele in both forms. Allele frequencies at *sMDH-B1,2** isoloci were estimated from the maximum likelihood procedure of Waples (1988). Allele frequencies at these isoloci were not significantly different between dwarfs and normals (first locus, G statistic = 3.15, $P > 0.05$; second locus, G statistic = 0.38, $P > 0.05$).

Locus and allele	Dwarf	Normal
*G3PDH-1**		
0	0.69	0.69
−85	0.31	0.31
*sMDH-B1,2** isoloci		
First locus		
100	0.27	0.39
120	0.73	0.61
Second locus		
100	0.80	0.76
120	0.20	0.24

showed quite low genetic variability, lacking the *G3PDH-1*(210)*, *sIDHP-3*(125)*, and *sIDHP-4*(118)* alleles which are common in other Ontario lake whitefish populations. The average expected heterozygosity was only 0.04 for both dwarfs and normals; allozyme genetic diversity was much lower than in other nearby lake whitefish populations (Bodaly et al. 1992).

TABLE 2. Definition and frequency distribution of mtDNA haplotypes, sample sizes, and estimated nucleon diversity index for dwarf and normal lake whitefish in Como Lake. Composites represent the following order of restriction enzymes: *Ava*I, *Ava*II, *Hinc*II. Corresponding fragment patterns are described in Bernatchez and Dodson (1991) except for *Ava*II "M" which differs from "A" by a single site loss. Other enzymes were fixed for fragment pattern "A."

Haplotype	Dwarf	Normal
1 AAA	30	35
2 AAB	1	8
3 AFA	0	1
4 CAA	4	0
5 AMA	1	0
Total	36	44
Nucleon diversity	0.30	0.34

The alleles present in both lake whitefish forms were those that are either fixed or common in lake whitefish that survived Wisconsin glaciation in the Mississippi, Atlantic, and Acadian glacial refugia (Bodaly et al. 1991b, 1992).

MtDNA Analysis

Five mtDNA haplotypes were observed among the 80 lake whitefish analyzed (Table 2). All haplotypes were only slightly differentiated, differing by one or two restriction sites. The first four corresponded to haplotypes 1, 17, 23, and 24, respectively,

as described in Bernatchez and Dodson (1991). Haplotype 5 had not been observed previously and differed from haplotype 1 by a single *Ava*II site loss. Three haplotypes (3, 4, 5) have been observed only in Como Lake lake whitefish. mtDNA diversity values were intermediate between values observed for Mississipian and Atlantic populations (Bernatchez and Dodson 1991). Significant heterogeneity in the distribution of mtDNA haplotypes was revealed by the likelihood ratio chi-square test ($G^2 = 14.099$, $P = 0.007$, df = 4).

Meristic Counts and Morphometric Measurements

The distribution of sexes in the samples was 25 male and 26 female dwarfs and 26 male and 24 female normals. Two-way analysis of variance (form and sex as levels) indicated that only lateral line scales, anal rays, anal length, caudal peduncle depth, and pelvic fin length differed between sexes when the effects of form were held constant. Within forms, only lateral line scales were significantly different between sexes for normals and dorsal fin origin, anal length, caudal peduncle depth, and pelvic fin length were different between sexes for dwarfs. These results, together with the nearly equal sex ratios within both forms, indicate that the results below are free from biases due to sexual differences.

Five of the nine meristic variables were significantly different ($P < 0.05$) between forms based on univariate analysis (Table 3). In all cases, the dwarf form exhibited a lower count than the normal form. Results obtained by nonparametric testing were similar. The multivariate test of the hypothesis of identity indicated a highly significant difference between the two forms ($P < 0.0001$) (Table 3). The variables contributing most substantially to the discriminant axis were those exhibiting the greatest univariate differences: ULS, LLS, LGR, UGR, and PEC in decreasing order. A posteriori classification accuracy using the discriminant function was high for the normal form (47 of 49, 96% of fish classified correctly) but lower for the dwarf form (20 of 29, 69% of fish classified correctly). This implied substantial overlap of the groups as was evident in the plots of discriminant scores (Fig. 2) and as was confirmed by principal components analysis.

Fourteen of 19 morphometric variables (residuals) were significantly different ($P < 0.05$) between forms based on univariate analysis (Table 4). The multivariate test of the hypothesis of identity indicated a highly significant difference between the two forms ($P < 0.0001$) (Table 4). A posteriori classification accuracy of the discriminant function was high for both forms (48 of 50, 96% correct for normals and 51 of 51, 100% correct for dwarfs). This implies very minimal overlap between forms in terms of body morphometry (Fig. 2), which is confirmed by principal components analysis and plots of scores on components.

Discussion

Our results indicate that lake whitefish in Como Lake show genetic differences and are therefore probably structured into two largely or wholly reproductively isolated subpopulations: dwarfs and normals. This conclusion is supported by significant differences in the frequency of mtDNA haplotypes between the two forms. Morphological differences observed also imply genetic differences between the two forms. Morphological variation in fish, both meristic and morphometric, is known to be influenced by both genetic and environmental factors. Therefore, the two forms differ in the genetic background of these characters, or in environments occupied at least during some critical time during development, or in both of these aspects. Because the two forms appear to spawn at the same time and together in at least some spawning locations (Bodaly et al. 1991a) and are therefore exposed to the same environment during development, genetic differences between them are probably at least partly responsible for the observed morphological differences. And dwarf and normal lake whitefish clearly exhibit different life histories with different growth rates, reproductive ages, and generation lengths. Parallel life history variation in Arctic char (*Salvelinus alpinus*) has been shown to have a genetic basis (Svedäng 1990). It seems likely that genetic differences also underly the life history differences between the Como Lake dwarf and normal lake whitefish.

The sympatric forms in Como Lake appear to have diverged to a lesser degree than other sympatric lake whitefish populations, which show divergence in allele frequency distributions and time and/or place of spawning in addition to morphological and life history characteristics (Fenderson 1964; Lindsey et al. 1970; Kirkpatrick and Selander 1979; Bodaly et al. 1988, 1992). This suggests that the divergence of the two forms is relatively recent. Furthermore, reproductive isolation may be incomplete and the two forms may still be exchanging genes. Because the two lake whitefish forms in Como Lake appear to spawn in the same place at the same time, differences in spawning behaviour, perhaps acting in concert with selection against hybrids or hybrid sterility, are most likely the basis for their reproduc-

TABLE 3. Meristic comparison of dwarf and normal lake whitefish in Como Lake. ns = nonsignificant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

Variable	Mean		*t*-test significance	Standard discriminant coefficient	Unstandardized discriminant coefficient
	Dwarf	Normal			
LLS	74.7	77.7	***	0.549	0.177
SPS	7.8	7.9	ns	0.237	0.617
ULS	9.5	10.2	***	0.770	1.373
DRC	11.6	11.7	ns	0.205	0.335
ARC	11.9	12.2	ns	0.067	0.094
PEC	16.3	16.7	*	0.024	0.033
PVC	11.3	11.5	ns	0.090	0.171
UGR	7.9	8.2	*	0.283	0.500
LGR	16.4	16.7	*	0.320	0.493
Constant					−51.877
λ				0.885	

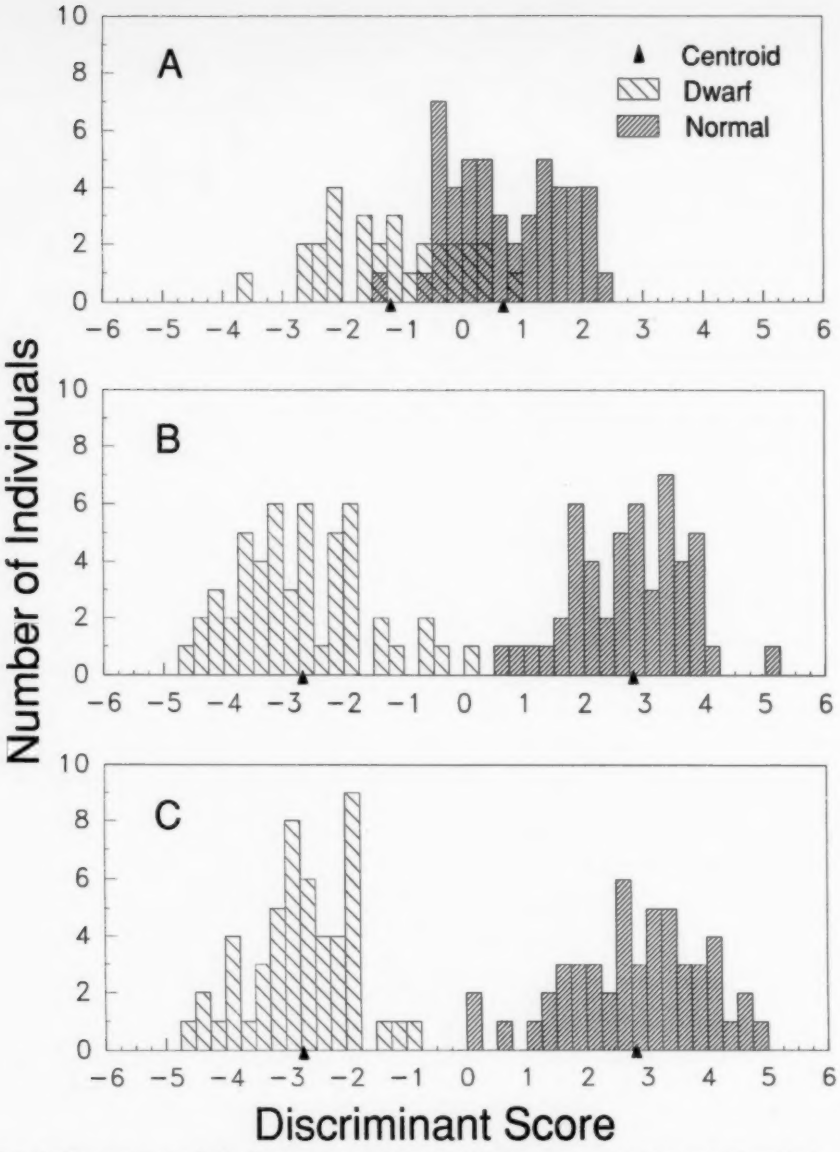

FIG. 2. Discriminant analysis of (A) meristics, (B) ratios of morphometric variables, and (C) residuals.

tive isolation. The two forms may have preferences for different sizes of mates, similar to the presence of alternative reproductive strategies in landlocked and anadromous sockeye salmon (*Oncorhynchus nerka*) (Foote 1988). Selection will tend to favour more complete reproductive isolation if hybrid individuals are less fit than either of the parental types. Size preferences by spawning individuals can provide the explanation for both a mechanism of reproductive isolation following allopatric divergence and a mechanism of reproductive isolation for sympatric divergence. Further studies on spawning biology are necessary to establish the mechanism for reproductive isolation between the dwarf and normal lake whitefish in Como Lake.

Partial reproductive isolation also accounts for the distribution of differences between the two forms. A low level of nuclear gene flow can prevent differences in isozyme frequencies. However, due to the maternal inheritance of mtDNA, nuclear genes could be passed from one form to the other without flow of mtDNA. Given a genetic basis for the size differences between forms, morphological differences may also be preserved in the face of gene flow.

Regarding the mode of origin of the Como Lake sympatric lake whitefish forms, there is both precedent and evidence which would tend to favour both of the contrasting hypotheses: recent divergence (i.e. postglacial) either sympatrically or during deglaciation and allopatric divergence in separate glacial refugia followed by secondary contact. Bodaly et al. (1992) examined sympatric lake whitefish populations in a number of Canadian lakes, utilizing allozyme allele frequencies, and concluded that the data best supported postglacial origins for such pairs. Allozyme data are, however, not definitive in determin-

TABLE 4. Morphometric comparison of dwarf and normal lake whitefish in Como Lake. Sig. = univariate significance with ns = nonsignificant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; STD = standardized, UNSTD = unstandardized.

	Residual			Ratio (× 100)			Discriminant coefficients			
	Mean			Mean			Residual		Ratio	
Variable	Dwarf	Normal	Sig.	Dwarf	Normal	Sig.	STD	UNSTD	STD	UNSTD
POL	0.115	−1.311	***	5.3	5.5	*	0.030	0.029	0.389	78.721
OOL	0.719	−0.416	***	5.9	4.7	***	0.076	0.112	−0.715	−195.629
PSL	0.149	−0.381	*	11.6	11.5	ns	0.239	0.191	0.092	15.195
TTL	−4.127	2.861	***	22.0	23.8	***	0.661	0.270	0.567	47.975
DOL	1.679	1.211	ns	11.6	11.6	ns	0.094	0.042	−0.170	−16.102
LUL	−1.313	4.309	***	19.2	19.8	ns	0.226	0.057	0.205	11.205
ANL	0.719	−0.699	***	9.3	9.5	ns	−0.326	−0.175	0.198	21.166
CPL	−3.391	−2.701	ns	11.7	11.1	*	0.571	0.218	−0.222	−16.381
HDD	0.909	−0.199	***	10.1	9.7	**	−0.045	−0.041	0.135	24.138
BDD	−2.047	−1.414	ns	22.7	24.0	***	0.197	0.088	0.412	36.672
CPD	0.481	0.462	ns	7.5	7.6	ns	0.287	0.306	0.111	23.637
IOW	0.028	−1.485	***	5.9	5.8	ns	−0.470	−0.497	0.017	3.327
MXL	1.401	−0.181	***	6.7	6.1	***	−0.388	−0.582	−0.271	−74.194
MXW	0.283	0.116	*	2.1	2.1	ns	0.226	0.599	0.102	56.318
PCL	2.714	−1.009	***	18.7	18.2	**	−0.306	−0.164	0.007	0.776
PVL	2.811	0.321	***	19.3	18.5	***	−0.172	−0.113	−0.091	−10.517
ADL	−0.518	2.430	***	6.8	5.9	***	0.489	0.199	−0.549	−45.937
GRL	0.169	−0.265	***	2.5	2.5	ns	−0.090	−0.191	0.222	96.332
LAL	0.063	0.041	ns	8.5	7.8	***	0.567	0.580	−0.309	−67.572
Constant								0.722		−8.660
λ							7.809		7.676	

ing racial origin for sympatric pairs which possibly involve the Mississippi, Atlantic, and Acadian glacial refuge races, such as those in Como Lake, because these races are less differentiated allozymically than other adjacent races such as the Mississippi and Bering (Bodaly et al. 1992). The allozyme evidence does tend to favour a recent origin for the Como Lake forms on the basis of their small genetic divergence and quite low heterozygosities. Low heterozygosities may indicate genetic drift during a founder event immediately following deglaciation and subsequent sympatric divergence. Numerous other salmonid species appear to have diverged locally into sympatric forms, including Arctic char, brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), brook trout (*Salvelinus fontinalis*) Atlantic salmon (*S. salar*) (see Foote et al. 1989 for a review and references), and, in the coregonid fishes, vendace (Vuorinen et al. 1981).

In contrast with this support for an hypothesis of recent, sympatric divergence, Bernatchez and Dodson (1990a, 1991) concluded, on the basis of mtDNA analyses, that sympatric dwarf and normal lake whitefish in the Allegash basin, Maine, have an allopatric origin representing two separate glacial races, namely the Atlantic and Acadian races. For the sympatric forms in Como Lake, the mtDNA evidence suggests an allopatric origin for the two morphs in the Mississippi and Atlantic refugia. One haplotype (No. 2, Table 2), which occurs predominantly in the normal morph, is characteristic of lake whitefish populations found in southeastern Quebec and is indicative of fish that probably survived the last continental glaciation in an Atlantic refugium (Bernatchez and Dodson 1991). An hypothesis of allopatric origin of the Como Lake forms would predict that the normal size form in other Ontario lakes supporting sympatric morphs (i.e. Opeongo and hopefully others to be discovered) would, like the Como Lake normal form, also show mtDNA haplotype 2. Also, haplotype 2 should be found in a corridor between southern Quebec and Como Lake (and beyond), following a dispersal route utilizing glacial Lake Nippissing I (Underhill 1986).

These results have implications for the management and preservation of these two forms. The phenomenon of size variation in coregonids that results in sympatric forms as in Como Lake is not unique but is relatively uncommon. Therefore, management steps should be taken to ensure preservation of both forms of lake whitefish. Dwarf lake whitefish survive only in the absence of ciscoes (Bodaly et al. 1988). The introduction of ciscoes into Opeongo Lake, Ontario, appears to have resulted in a reduction in the abundance of the dwarf lake whitefish (Bodaly et al. 1991a). Also, the spread of rainbow smelt (*Osmerus mordax*) into lakes in Maine has apparently reduced the normal form of lake whitefish in lakes supporting two forms (L. Bernatchez, unpubl. data). Therefore, preservation of sympatric lake whitefish forms will require vigilance against the introduction of new species into lakes such as Como Lake.

Ackowledgments

We thank Messrs. Lee Burke and Ken Woodard of Como Lake Resort and J. Seylor and N. Dawson of the Ontario Ministry of Natural Resources for assistance in the field. Additional sampling assistance was provided by Yu. Reshetnikov, H. Lehtonen, and V. Macins. T. Carmichael, F. Colombani, R. Fudge, J. Johnson, and M. Koshinsky assisted in many ways in the laboratory. We thank C. J. Foote for helpful discussions. J.A.V. was supported by the Finnish Academy (grant No. 8001114) during his leave at the Freshwater Institute.

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Changes in Erythron Organization during Prolonged Cadmium Exposure: An Indicator of Heavy Metal Stress?

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Houston, A., S. Blahut, A. Murad, and P. Amirtharaj. 1993. Changes in erythron organization during prolonged cadmium exposure: an indicator of heavy metal stress? Can. J. Fish. Aquat. Sci. 50: 217–222.

Changes in erythron organization were examined in goldfish (*Carassius auratus*) exposed for 3 wk to 5, 15, and 25% of 240-h Cd LC50. In a subsequent, more extended study, specimens were first rendered acutely anemic by immersion in phenylhydrazine HCl, allowed to recover in Cd-free water or in concentrations equivalent to ~5 or ~11% of LC50, and sampled after 2, 5, 8, and 11 wk. Total, dividing, and degrading or karyorrhectic cell numbers were recorded, as were cell length, one-sided surface area, and form factor. In fish exposed to ~11% LC50, total cell numbers and the incidence of cell division fell, while karyorrhexis increased. Dividing to total cell ratios declined with time; karyorrhectic to total and karyorrhectic to dividing cell ratios increased. Variations in cytomorphology suggested slower cell maturation at both metal concentrations. Detection of heavy metal stress in fishes by this form of hematological assessment appears feasible.

On a étudié les modifications dans l'organisation des organes érythrocytaires chez le cyprin doré (*Carassius auratus*) exposé pendant trois semaines à des concentrations équivalent à 5, 15 et 25 % de la CL50 de 240 h du Cd. Au cours d'une étude ultérieure plus poussée, on a d'abord provoqué une anémie sévère chez les spécimens en les immergeant dans du chlorhydrate de phénylhydrazine, puis en les laissant se rétablir dans une eau exempte de Cd ou à des concentrations équivalent approximativement à 5 ou 11 % de la CL50; on a ensuite prélevé des échantillons après 2, 5, 8 et 11 sem. Le nombre total de cellules ainsi que le nombre de cellules en voie de division et de cellules en caryorrhexie ou en voie de dégradation ont été notés, en plus de la longueur des cellules, de la surface érythrocytaire d'un seul côté et du facteur morphologique. Chez les poissons exposés à une concentration d'environ 11 % de la CL50, le nombre total de cellules et l'incidence de la division cellulaire diminuent tandis que la caryorrhexie augmente. Le rapport entre le nombre de cellules en voie de division et le nombre total de cellules diminue en fonction du temps; cependant, il y a augmentation du rapport entre les cellules en caryorrhexie et le nombre total de cellules ainsi que du rapport entre les cellules en caryorrhexie et les cellules en voie de division. Les variations cytomorphologiques laissent entrevoir un ralentissement de la maturation cellulaire pour les deux concentrations de métal à l'étude. Il semble donc possible d'évaluer le stress en présence de métaux lourds chez les poissons à l'aide de ce type d'évaluation hématologique.

Received December 19, 1991
Accepted July 7, 1992
(JB342)

Reçu le 19 décembre 1991
Accepté le 7 juillet 1992

The teleostean erythron is a dynamic entity whose ontogenic composition, and thus O_2 transport capacity, reflects the interaction of several processes. Prominent among these are cell formation by pronephric and splenic erythropoiesis or peripheral cell division, maturation, aging, and cell breakdown or karyorrhexis (Murad et al. 1990; Houston and Murad 1992). In complex multicomponent systems, factors that differentially influence the rates of the contributing activities inevitably alter overall status to some greater or lesser extent (Grodins 1963; Riggs 1963). This being the case, a pollutant exerting differential effects on any aspect of erythropoiesis and/or ontogenic progression would be expected to affect the composition of the circulating red cell population.

In the specific case of Cd, chronic exposure to nonacute environmental levels commonly leads to anemia (Johansson-Sjobeck and Larsson 1978; Haux and Larsson 1984; Sjobeck et al. 1984; Gill and Pant 1985, 1986). This cannot be attributed to hemodilution (McCarty and Houston 1976) but is well correlated with extensive lesioning of hematopoietic tissues (Stromberg et al. 1983) and impaired erythropoietic capacity (Houston and Keen 1984). Detectable changes in erythron organization would therefore be anticipated at Cd concentrations below the acutely lethal. To test this hypothesis, red cell populations of goldfish (*Carassius auratus*) exposed under laboratory conditions to sublethal levels of Cd were periodically characterized for up to 11 wk.

Materials and Methods

Origin and Maintenance of Goldfish

Goldfish (mean weight ± 1 SEM 6.6 ± 1.71 g) were obtained from a local commercial supplier (Hartz Mountain, Rexdale, Ontario). Upon arrival, they were surface disinfected by immersion in 10^{-5} $KMnO_4$ and transferred for 2 wk to holding troughs maintained at 25.0 ± 0.2°C, a widely accepted value for the thermal optimum of this species (Houston 1982). Supplementary aeration maintained O_2 levels at or above 6.8 mg/L (approximately 80% saturation). Photoperiod hoods provided water surface light intensities of 90–120 lx on a 12 h light : 12 h dark photoperiod regime initiated at 08:00.

Animals were fed once daily to satiation on commercial pellets, and excess food and fecal material was removed regularly. As judged by general activity, feeding behavior, and the absence of obvious disease symptoms, they remained healthy throughout the study period.

Experimental Protocol

The Cd content of dechlorinated St. Catharines city water is relatively low (<10 μg/L). Concentrations of the two other principal local heavy metal contaminants, Zn and Cu, are less than 50 μg/L and below reported threshold toxicities for most freshwater species. However, pH (7.4–8.0) and total hardness (135–150 mg/L as $CaCO_3$) are such that added Cd is rapidly lost as $CdCO_3$, $Cd(OH)_2$, and other precipitates. Earlier studies involving initial Cd loads of 1–60 mg/L, for example, revealed exponential decreases in dissolved Cd to negligible levels within 24–48 h (McCarty et al. 1978). Hardness and conductivity rose in proportion to the amount of Cd added, while total alkalinity and pH exhibited transient decreases. In the latter case, changes of as much as 1.0–1.2 pH unit were observed.

To avoid the complications inherent in time-variable conditions, water of reduced hardness was used. This was prepared by dilution of dechlorinated and aged city water with glass-distilled water and had the following characteristics: total hardness, 20–22 mg/L as $CaCO_3$ total alkalinity, 14–18 mg/L as $CaCO_3$; conductivity, 15–25 siemens/cm; pH, 7.4–7.8; $[Cd^{2+}]$, <2 μg/L. Little precipitation occurred in this medium following Cd addition, and changes in water quality were markedly reduced (McCarty et al. 1978).

In initial trials, fish were transferred in water to 100-L fibreglass test tanks containing artificial soft water. Other conditions were as described. One week was provided for recovery from transfer stresses. Analytical-grade $CdCl_2$ dissolved in glass-distilled water was then added to bring tank concentrations to 90, 270, and 445 μg/L, i.e. approximately 5, 15, and 25% of the softwater 240-h LC50 for this species (McCarty et al. 1978). Water levels were checked daily and adjusted to compensate for evaporation. Determinations by Beckman Spectrascan-5 flame emission spectrophotometry revealed little fluctuation with time. Following exposure to these conditions for 3 wk, all specimens were sampled. This schedule was repeated to provide 10–12 animals at each concentration.

A second, longer term study was then undertaken. Following acclimation to the conditions described, groups were brought to 5°C at a rate of ~1°/d. To ensure comparability of hematological status, all animals were rendered anemic by immersion in 1 mg/L solution of phenylhydrazine HCl for 24 h in foil-shielded, aerated tanks (Houston et a. 1988). Following exposure, they were returned to their original tanks, treated with chlorotetracycline, and held at 5°C for 2 wk. Previous studies (Houston et al. 1988; Marinsky et al. 1990) have shown that this is sufficient to bring red cell numbers, hemoglobin, and hematocrit to minimal values. Erythropoiesis was induced by increasing temperature to 25°C at a rate of 1–2°/d.

As in the original trial, it was intended to expose fish to ~0, ~5, and ~15% of LC50. Subsequent analyses revealed, however, that mean Cd concentrations were actually 84.5 and 187 μg/L, rather than 89 and 267 μg/L, i.e. 4.7 and 10.5% of the 240-h LC50. These are subsequently referred to as 5 and 11%. Sampling was carried out after exposures of 2, 5, 8, and 11 wk and the schedule repeated to give the sample numbers subsequently reported.

Sampling

To avoid possible induction of artifacts by chemical anesthesia (Korcock et al. 1988), specimens were stunned with a spring-driven impact tool before sampling and killed immediately afterward by transection of the medulla. Samples were drawn from the caudal vessels into dipotassium EDTA treated collection tubes and held on ice before use. All hematological procedures have been described in detail (Houston 1990). Erythrocyte counts were carried out by hemocytometer. Blood smears were prepared, air-dried, fixed in methanol, and stained with Leishmann–Giemsa. To estimate the abundances of karyorrhectic and dividing cells, approximately 1000 cells were identified for each fish. Preliminary studies were carried out to determine the precision of the counting method. A minimum of four smears were prepared from a series of specimens. Several counts were carried out on each smear using a variety of methods for the selection of microscope fields. Results were then evaluated in terms of the effect of field selection method and number of replicate counts on mean and standard error. This indicated a minimum requirement of at least two smears per specimen and three fields per slide selected from a predesignated grid system using a random numbers table.

Cell deformation during smear prepartion can lead to distortion and difficulty in subsequent cell identification. Because of the particular sensitivity of thrombocytes to physical distortion, only slides with thrombocytes of normal morphology (Ellis 1977) were scored.

Categorization of the erythron on the basis of differential smear counting presents problems principally in relation to juvenile cells. As visualized by Romanowsky staining procedures, the normal mature goldfish erythrocyte is symmetrically ellipsoidal. The ovoid, centrally positioned nucleus stains intensely and homogeneously blue–purple and the cytoplasm a pinkish orange. Nuclear chromatin and interchromatin regions and cytoplasmic inclusions are only occasionally seen. Recent studies have provided information on the cytomorphic characteristics of juvenile and mature red cells in this species (Houston and Murad 1991; Murad and Houston 1992). Thus, development of [^{3}H]thymidine-labelled cell cohorts in fish recovering from phenylhydrazine-induced anemia indicated that mature cells are characterized by mean major axis and one-sided surface area values, respectively, of 11.2 μm and 68.5 μm^2 or more. Axis ratio and form factor were 0.716 and 0.912 or less, respectively. Corresponding values for juvenile cells were major axis <9.2 μm; one-sided surface area ≤50 μm^2, axis ratio >0.774, form factor >0.938.

Thrombocytes, lymphocytes, monocytes, and the various granulocyte types can be distinguished from mature erythrocytes on the basis of these variables, nuclear position, and nuclear morphology. While disagreement exists with respect to the classification of cells within this general category, the most common types can be differentiated from erythrocytes on the basis of cytoplasmic granulation and staining properties. Lymphocytes, monocytes, and thrombocytes can usually be distinguished from juvenile red cells on the basis of their cytomorphic characteristics, nuclear position, nucleus to cytoplasm ratio, and cytoplasmic staining properties. However, small numbers cannot be identified with certainty. These were recorded as unidentified cells and were not included in subsequent calculations.

In erythrocytes undergoing karyorrhexis, cell and nuclear profiles typically shift from ovoid to round (Murad et al. 1990). Nuclear staining intensity is reduced and numerous irregular, vacuolelike structures are evident. Single or multiple irregular nuclear protrubances are sometimes present. Cytoplasmic staining intensity is reduced and extensive vacuolization occurs. Enlargement of the nucleus is accompanied by reduction in cytoplasmic volume, and in the final stages of breakdown, only what appears to be a naked, swollen nucleus remains. Cells meeting these criteria do not incorporate radiothymidine.

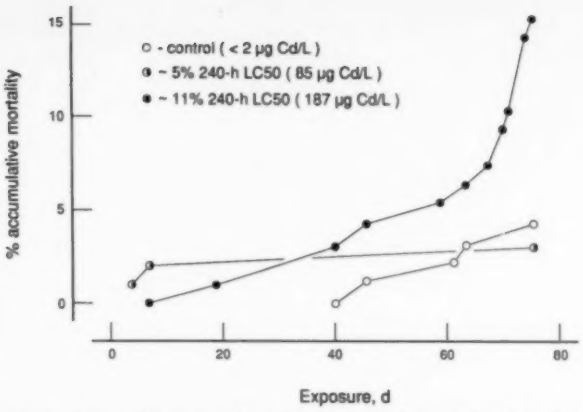

FIG. 1. Accumulative mortality (% experimental population) in goldfish chronically exposed to ~0% (<2 µg/L), ~5% (85 µg/L), and ~11% (187 µg/L) of 240-h Cd^{2+} LC50.

Dividing cells display nuclear elongation with modest central construction (Murad et al. 1990, 1992). This is followed by cellular elongation and constriction. Cell segments attenuate, move apart, and eventually separate. Their nuclei then shift centrally and cytoplasm volume increases. A taillike projection persists for an unknown period. Such cells take up both [^{3}H]thymidine and ^{55}Fe (A. Murad and A. H. Houston, unpubl. obs.).

Image analysis was employed to examine the effects of exposure to Cd on several cytomorphic variables. A Cohu Solid State 4815-2000 camera linked to a Nikon 810326 microscope traced images within predefined shade limits, conveying the data to an IBM PCAT microcomputer. An Imaging Research Inc. software program translated trace data to values for cell and nuclear maximum chord (length) and maximum perpendicular chord (width), one-sided surface area, and form factor ($4\pi \cdot \text{area/perimeter}^2$). Changes with time were taken as indicative of maturation rate. A minimum of 36 cells were measured from randomly selected fields on one randomly selected smear from each specimen.

Results

Mortality

Postexposure mortality in phenylhydrazine-treated fish was 3%, well below the levels commonly encountered following intraperitoneal administration of, or immersion in, this hemolytic agent (Cameron and Wohlschlag 1969; Smith et al. 1971; Chudzik and Houston 1983; Houston et al. 1988). We believe that this may be attributable to the antibiotic treatment.

No mortalities were observed during initial 21-d exposures to 90, 270, and 445 µg Cd/L. Similarly, over the course of the longer term study, accumulative mortality in the control and 5% groups was low (Fig. 1). However, among fish exposed to 11% of LC50, mortality rose in near-exponential fashion after an initial lag period, accounting for 15% of the test group by week 11.

General Hematology

Prior to phenylhydrazine treatment, hematological indices were comparable with those previously reported for this species, i.e. red cells 1.51 ± 0.107 × 10^6/mm^3, hemoglobin 6.9 ± 0.56 g/dL (Anthony 1961; Houston 1980; Murad et al. 1990). Separating cells were present but not common. At 7.18 ± 1.89% of the total erythrocyte population, karyorrhectic cells were relatively abundant. Following exposure to phenylhydrazine, but prior to increase in temperature, red cell numbers had fallen to 0.12 ± 0.37 × 10^6/mm^3 (−92.1%). Karyorrhectic cells declined to 1.15 ± 0.15% of the total, while separating cells accounted for 0.38 ± 0.18% of the red cell population. Again, these were in reasonable agreement with earlier observations (Houston et al. 1988).

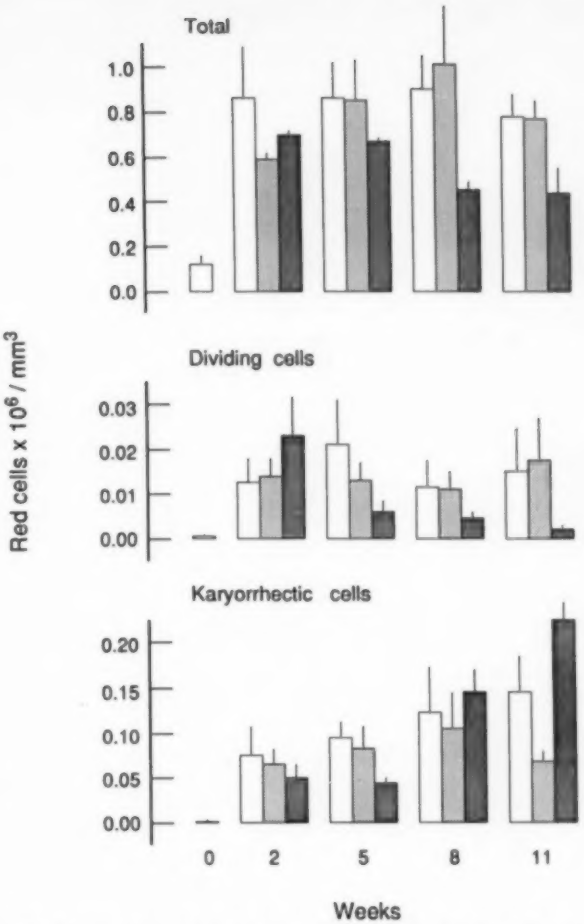

FIG. 2. Total, dividing, and karyorrhectic red cells in goldfish chronically exposed to ~0% (<2 µg/L, open), ~5% (85 µg/L, lightly cross-hatched), and ~11% (187 µg/L, densely cross-hatched) of 240-h Cd^{2+} LC50. Values reported as mean ± 1 SEM.

In the first test the incidence of karyorrhexis was significantly elevated ($P < 0.05$ to $P < 0.01$) at all Cd concentrations, such cells comprising 0.36 ± 0.5, 3.16 ± 0.42, 1.29 ± 0.39, and 1.30 ± 0.18% of cells in the control, 5, 15, and 25% LC50 groups, respectively. Corresponding values for dividing cells were 0.34 ± 0.06, 0.38 ± 0.07, 0.36 ± 0.05, and 0.45 ± 0.07%. Of these, only that from the 25% LC50 group differed significantly ($P < 0.05$) from the control group.

Variations during the subsequent, more prolonged study were consistent with those initially noted at comparable times (Fig. 2). As would be anticipated, total cell numbers increased

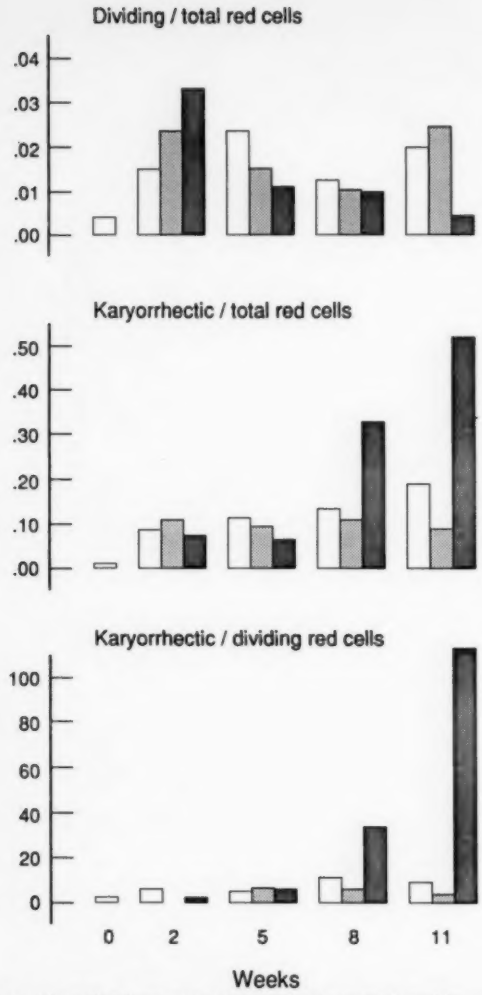

FIG. 3. Mean dividing to total, karyorrhectic to total, and karyorrhectic to dividing cell ratios in goldfish chronically exposed to ~0% (<2 μg/L, open), ~5% (85 μg/L, lightly cross-hatched), and ~11% (187 μg/L, densely cross-hatched) of 240-h Cd^{2+} LC50.

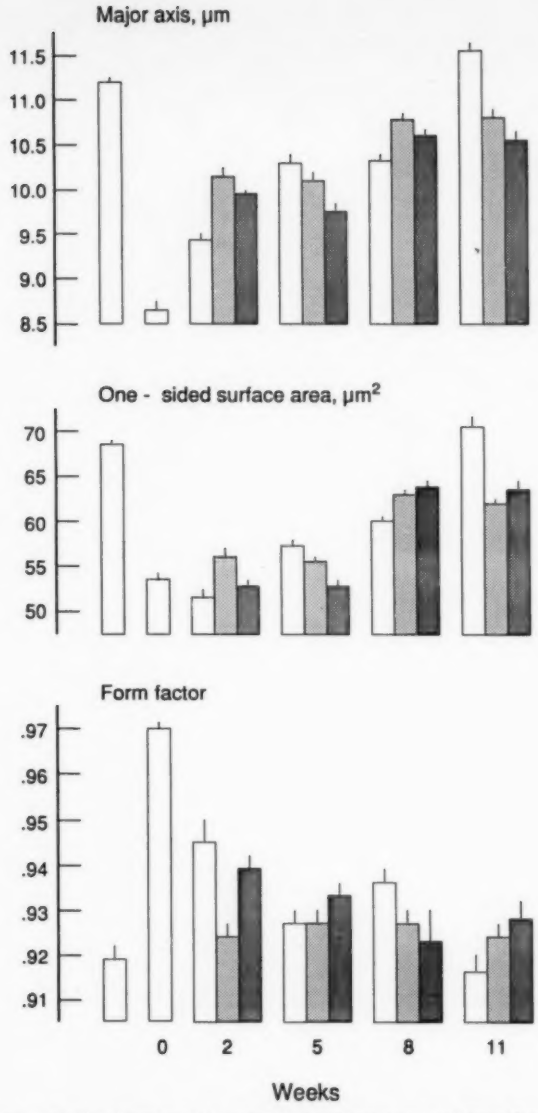

FIG. 4. Red cell major axis, one-sided surface area, and form factor in goldfish chronically exposed to ~0% (<2 μg/L, open), ~5% (85 μg/L, lightly cross-hatched), and ~11% (187 μg/L, densely cross-hatched) of 240-h Cd^{2+} LC50. Data reported as mean ± 1 SEM.

significantly in all groups during the first 2-wk period. No significant differences between groups were evident. During the ensuing 9 k, the control and 5% groups did not change or differ significantly. By contrast, in the 11% group, cell numbers declined steadily and during weeks 8 and 11 fell significantly ($P < 0.05$) below those in both the control and 5% groups.

Much the same was true of the incidence of cell division. Dividing cell abundances were elevated after 2 wk in both the control and 5% groups but exhibited no notable changes thereafter. In the fish maintained at 11% LC50, their numbers fell to near-negligible levels after 11 wk.

The occurrence of karyorrhexis also differed with treatment. Although there was initially little difference among the test groups, karyorrhectic cell numbers increased steadily in the 11% group and by the final sampling period were significantly in excess of those seen in the control and 5% groups.

Expressed as relative rather than absolute cell numbers, these differences were even more evident (Fig. 3). This was particularly the case with the 11% group. Thus, the ratio of dividing to total cell numbers fell from 0.033 after 2 wk to 0.005. Similarly, karyorrhectic cells relative to total cell numbers rose from an initial value of 0.073 to 0.328 after 8 wk and 0.518 at the final sampling period. The mean ratio of karyorrhectic to dividing cells, which ranged from 4.6 to 10.7 in control and 5% animals, reached a final value of 112.6 in the 11% group.

Cytomorphology

Before induction of anemia, cytomorphic characteristics were similar to those previously reported for goldfish (Houston and Murad 1992). Following treatment, but prior to exposure to Cd, mean cell major axis was reduced (Fig. 4). So was surface area, while form factor was sharply higher. Such characteristics are consistent with a population of preponderantly juvenile cells.

Under Cd-free conditions, values for all variables increased steadily, reaching levels not significantly different from those of pretreatment fish after 11 wk. In both exposure groups, however, final values for major axis and surface area were well below those characterizing the control group. Similarly, mean form factor in both Cd groups exceeded that of control fish and, in the case of the 10% group, the difference was significant ($P < 0.05$).

As an approximation of cell age distribution, percentages of cells having major axes and surface areas less than and form factors greater than those observed before phenylhydrazine treatment were calculated (Table 1). In all groups, these pointed to steadily maturing cell populations. It will be evident, however, that control and test groups differed with respect to length and surface area during weeks 8 and 11. Such values suggest some retardation of the maturation process.

Discussion

Material flow through aquatic food chains commonly leads to bioconcentration at higher trophic levels. Because of this, and the intimacy of aquatic organism – environment interactions, the occupants of these levels can be regarded, in some respects at least, as integrators of habitat conditions. This being the case, it has been suggested that development of reliable means for the detection and quantification of stress states in fishes might provide a basis for assessment of overall environmental quality. Beitinger and McCauley (1990) and Wedemeyer et al. (1990) have recently reviewed the growing literature in this field, systematizing and evaluating approaches to stress assessment in terms of their biochemical, physiological, and organismic manifestations.

Responses to stress are commonly termed primary, secondary, and tertiary. Of these, primary responses stem from direct stressor effects at the cell molecular level, e.g. on membrane organization and permeability, substrate activation state, the activities of specific enzyme systems, etc. Induction of endocrine responses is also regarded as primary although these responses presumably reflect more primary events at the cellular level. Secondary responses stem from primary effects and are often seen as deviations from the norm in various regulated activities: osmotic, ionic, and acid–base regulation, glucostasis, immunological competence, erythron status, acclimatory capacity, and similar functions. Effects at the tertiary or whole-organism level include a wide range of activities such as overall metabolism, growth, competitive and reproductive abilities, mortality, etc.

The hematological changes observed in stressed fish presumably reflect more primary events such as stressor-induced variations in specific enzyme activities and circulating levels of catecholamines, steroids, and other stress-related hormones, i.e. they are derivative or secondary responses. As is the case with many such effects, the pathway(s) by which primary events are translated to detectable responses are not well understood for the most part. Nevertheless, their occurrence is undoubted.

In concept, the utilization of hematological variables for stress detection is attractive. Unit costs for the determination of the common primary and secondary indices (red and white cell counts, hematocrit and leucocrit, total hemoglobin, mean erythrocyte volume, and hemoglobin) are relatively low, while through-put is normally high. The procedures involved do not require unusual facilities or expertise. However, as currently employed, they are insensitive. The changes most commonly reported (anemia, polycythemia, leucopenia, and leucophilia) are frequently seen only under circumstances of acute response to gross challenge (Wedemeyer et al. 1990; Sorensen 1991).

Nevertheless, recent studies indicate that the blood cell system offers scope for more subtle employment. Once regarded as relatively stable, the erythron is emerging as a dynamic, responsive entity (Houston 1990). Respiratory stresses as diverse as reduced blood O_2-carrying capacity, transient exposure to hypoxic conditions, and transient thermal elevation of O_2 demand invoke a common pattern of response. This is characterized by increases in rates of erythropoiesis, cell maturation, division of circulating juvenile cells, and karyorrhexis (Murad et al. 1990; Houston and Murad 1992). As a result the age distribution of the red cell population shifts in favor of immature cells. This is critically important. Only two factors can be adjusted at this level: hemoglobin–O_2 affinity and blood O_2-carrying capacity (Houston 1980; Powers 1980; Weber 1983). These are a function of (1) the types and amounts of specific hemoglobin isomorphs present and (2) cell levels of affinity-modulating solutes such as ATP, GTP, H^+, Cl^- and Mg^{2+}. In turn, these appear to be related to the conditions under which juvenile cells mature (Byrne and Houston 1988; Marinsky et al. 1990). The coupling of new cell addition to elimination of some proportion of the preexisting cell population permits significant alteration in overall blood gas transport characteristics. In addition, a system of this kind offers the advantage of minimizing increases in overall cell numbers, and therefore viscosity and ultimately cardiac work requirements.

Our observations support the prediction that detectable, significant changes in erythron organization follow long-term exposure to Cd concentrations well below acutely lethal levels. Indeed, even total red cell count, a relatively insensitive index, was altered after 8–11 wk of exposure to only 10% of the 240-h LC50.

A number of factors may have contributed to observed changes. At any given moment the number of circulating red cells represents a balance between all processes adding cells to or removing them from circulation. The lymphomyeloid tissues of the pronephros and spleen are principal erythropoietic sites

TABLE 1. Relative abundances of erythrocytes having major axis (11.2 μm), one-sided surface area (68.3 μm^2), and form factor (0.919) values less than pretreatment values.

	Major axis			Surface area			Form factor		
Time	Control	5%	11%	Control	5%	11%	Control	5%	11%
post-PHZ[a]	98.3	—	—	95.8	—	—	93.3	—	—
2 wk	94.7	92.5	98.7	98.7	95.8	98.0	76.8	55.8	74.0
5 wk	86.7	83.3	90.7	86.7	83.3	90.7	67.3	60.0	70.6
8 wk	88.0	74.0	77.3	94.6	83.3	74.0	72.0	62.7	60.0
11 wk	73.3	66.7	70.7	52.0	83.3	76.0	48.7	58.7	67.3

[a]Fish sampled 2 wk following exposure to phenylhydrazine HCl.

of teleosts (Fange 1986). The damage to these tissues observed by Stromberg et al. (1983) and the inhibition of heme synthesis reported by Johansson-Sjobeck and Larsson (1978) presumably underlie demonstrated reductions in erythropoietic capability (Houston and Keen 1984).

Division of circulating cells was also significantly reduced. Apparently limited to immature erythrocytes (those possessing the organelles required for hemoglobin synthesis), division leads to cells able to take up and incorporate ^{55}Fe into hemoglobin (Murad et al. 1992). In addition, however, karyorrhexis also increased significantly. Consequently, it would seem reasonable to attribute observed decreases in cell numbers to reduced red cell formation and removal of existing, presumably senescent cells through increased karyorrhexis.

However, the effects of Cd were more obvious in relation to dividing to total, karyorrhectic to total, and particularly karyorrhectic to dividing cell ratios. All were markedly altered during even the relatively short-duration exposures employed in this study. Moreover, since essentially all heavy metal pollutants affect heme metabolism, cytoskeletal organization, and cell division and/or exert other rate-controlling or toxic effects (Sorensen 1991), these simple, commonly used hematological procedures may be of general utility.

It is worth noting in this context that changes in the absolute and relative abundances of leucocytes also follow exposure. Under generally similar conditions, total leucocyte numbers decreased from $44.92 \pm 0.72 \times 10^3$ to $22.52 \pm 2.32 \times 10^3$ cells/mm^3 after 3 wk at 25% LC50 (Murad and Houston 1988). Thrombocyte numbers remained approximately constant, while lymphocyte abundances declined and granulocyte numbers increased. As a result, the mean neutrophil to lymphocyte ratio rose more than fivefold from 0.087 to 0.478. Clearly, these as well as erythron variables may be useful indicators of stress.

Finally, since the erythrocyte and leucocyte variables exhibiting the largest changes are all ratios, blood smears prepared in the field by minimally traumatic sampling procedures for subsequent evaluation in the laboratory may suffice for diagnostic purposes.

Acknowledgements

The authors gratefully acknowledge the financial support provided by the Natural Sciences and Engineering Research Council of Canada through Ind. Op. Grant A6972 to A. H. Houston. Dr. R. W. McCauley provided valuable comment on the manuscript.

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Guide for Authors

Introduction

We welcome manuscripts reporting significant new information in fisheries and aquatic sciences. Manuscripts may concern cells, organisms, populations, ecosystems, or processes that affect aquatic production systems, and cover numerous disciplines including biology and ecology of marine and freshwater organisms, physiology, toxicology, genetics, limnology, oceanography, economics, disease, and management.

Manuscripts are selected for publication according to the extent and significance of new knowledge or ideas presented. Preference will be given to those whose experimental results emphasize causal mechanisms underlying observed phenomena rather than pure description and fact. Manuscripts should lead to identifiable conclusions or syntheses, which variously may amplify, modify, question, or redirect accumulated knowledge embodied in contemporary perceptions of a particular state of aquatic sciences. Their novelty must relate to more than the particular (a certain year, place, taxon, chemical compound) and their contribution to science must clearly be beyond the confirmatory state.

Manuscripts submitted should be as comprehensive as possible; research fragments are generally unacceptable. If a single paper cannot be produced, then closely related papers should be cross-referenced and submitted together.

The *Journal* considers the following types of contributions:

Articles: Studies of broad scope that are original contributions to science.

Reviews: Detailed critical appraisals of broad areas of investigation, particularly those that have developed rapidly in recent years.

Perspectives: Essays of opinion or hypothesis on aquatic science of concern to the professional and layperson. References are desirable; informal style is welcome.

Comments: Opinion on topics recently discussed in the *Journal*; preferred maximum length, 500 words.

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Submit manuscripts in triplicate to the Editor, *Canadian Journal of Fisheries and Aquatic Sciences*, Research Journals, National Research Council of Canada, Ottawa, Ontario, Canada K1A 0R6, accompanied by a covering letter that (1) states the main points and significance of the work, (2) avows that all coauthors fully participated in and accept responsibility for the work and that it is not being considered for publication elsewhere, (3) suggests potential referees, and (4) identifies other manuscripts containing the same or similar information. Original typescripts and figures (except halftones) are best retained by the author until requested. Packaging of manuscripts and illustrations should be robust enough to resist damage in transit.

Each manuscript is normally submitted to two referees for appraisal before final evaluation by an editor. However, the Editor will return unreviewed those manuscripts that do not fall within the *Journal's* editorial policy or scope.

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The *Council of Biology Editors Style Manual* (5th ed. CBE, 9650 Rockville Pike, Bethesda, MD 20814) is used as the authority in matters of form. The standards for spelling are either *Webster's Third International Dictionary* or the *Oxford English Dictionary* and *Le Grand Larousse encyclopédique*. Authors are responsible for consistency in spelling. Titles of periodicals are abbreviated as in *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103). Authors are responsible for ensuring the accuracy and completeness of their reference list. The *Journal* follows the names and spelling for fishes recommended in *A List of Common and Scientific Names of Fishes from the United States and Canada* (5th ed. 1991. Spec. Publ. No. 20, American Fisheries Society) and the gene nomenclature for protein-coding loci outlined in Shaklee et al. (1990. Trans. Am. Fish. Soc. 119: 2–15). All measurements must be metric, use SI symbols in most instances (exceptions noted in editorial in the *Journal*, Vol. 40, No. 12), and adhere to the *Canadian Metric Practice Guide* CAN3-Z234.1-79 (Canadian Standards Association, 178 Rexdale Boulevard, Rexdale, Ont. M9W 1R3).

Series titles should be avoided. An abstract is required for all manuscripts and should state concisely, in up to 175 words, what was done, found, and concluded.

Proofs are sent to the author, who is responsible for correcting errors in typesetting. *Alterations to the content must be avoided.*

Preparation of Manuscripts

General Guidelines

Movement of manuscripts through review channels and the editorial office is greatly expedited if manuscripts are prepared in *Journal* style and format. When in doubt about specific details, consult a current issue of the *Journal*. General guidelines follow.

Type the manuscript on white paper, preferably 8.5 × 11 in., on only one side of the page. Leave all margins at least 1 in. wide.

Type only the title, authors' names and affiliations, and related footnotes on the first page. Number all pages beginning with the title page, including those for tables and captions for illustrations.

Double-space all parts of the manuscript, including tables, captions for tables and figures, footnotes, and the reference list. Underline only material that is to be printed in italics.

Do not use all capitals anywhere in the manuscript.

Use the name-and-year system for literature citations.

Checklist for Manuscripts

Attention to the following questions will expedite appraisal of manuscripts by referees and editors.

Are the findings, interpretations, and conclusions adequately documented and relevant to the purpose of the study?

Are all the tables and figures needed, and organized to facilitate comparisons? Are there inconsistencies between tables and figures and the text, or within the text? Should some of the data be made available separately in a data or manuscript report or at a data repository?

If statistical analysis is included, is it subordinate to the research? When probability statements are made, are only the statistical tests cited and unnecessary statistical tables excluded?

Would any of the text be clearer if condensed? Are summary statements given at the beginning of sections and paragraphs, and are details in sections and paragraphs relevant to their topics? Does the organization of the manuscript follow logically from the statement of purpose in the introduction?

Does the *title* encompass the content of the report? Does the *abstract* give the essentials of the new knowledge? Is the *introduction* largely limited to the scope, purpose, and rationale of the study? Is review of the literature limited to defining the problem? Are details of *materials and methods* limited to what scientists need to understand the design of the study and to judge the adequacy of the data? Are generalizations from the *results* supported by the data provided? Are findings distinguished from inferences? Is the *discussion* limited to interpretation and significance of the findings?

The most common technical problems in submitted manuscripts are listed below. Authors can hasten the processing of their papers if they pay attention to these points during manuscript preparation.

1) Insert bibliographic strip before the abstract (Authors, date, title, Can. J. Fish. Aquat. Sci.).

2) Limit abstract to one paragraph of about 175 words.

3) Double-space all elements of the manuscript, including references, table captions, and figure legends.

4) Avoid exclusive use of capitals everywhere in the manuscript, including headings, table captions, and figure legends.

5) Underline only Latin names of organisms and certain statistical and mathematical notation to indicate italics.

6) Use correct SI symbols for units or measure in figures, tables, and text. Place a zero before the decimal for numbers less than unity.

7) Avoid ambiguous forms such as g $C/m^2/d$; use g $C \cdot m^{-2} \cdot d^{-1}$.

8) Identify test used to test statistical significance and give probability value. No reference is needed for common statistical tests.

9) In the text, generalize from tables and figures; avoid repeating all the details. Be sure each table and figure can stand on its own and is referred to in the text in numerical order. The captions should explain the purpose of the table or figure.

10) Include acknowledgments section at manuscript stage, not when page proofs arrive.

11) Provide the person's initials and mailing address when referring to personal communications.

12) Delete unnecessary references that do not apply directly to the problem.

13) Check references carefully against test citations and vice versa to ensure exact correspondence. Provide an availability statement for less easily retrieved material, e.g. available from Department of Economics, Simon Fraser University, Burnaby, B.C. V5A 1S6.

14) Delete commas between name and date in citations such as (Smith 1900) and do not underline "et al."

15) Replace 10" in table headings and figure axes with appropriate SI prefixes or use words (e.g. thousands).

16) Photocopies of figures, except halftones, are appropriate for review purposes. Send photographic prints or laser printouts of figures, or originals if they do not exceed 8.5 × 11 in., on request for publication. Use the same type of lettering throughout if possible.

Parts of the Manuscript

Organization

Organize the manuscript on the basis of the purpose or scope of the study as stated in the introduction. Ensure that the title and headings are in harmony with the statement of purpose.

Before writing any of the manuscript, list tentative headings in as few ranks as possible. Rework them until they appear to allow logical development for the reader; usually, chronological order is not effective. The findings will be more readily appreciated if methods, findings, and discussion are given in separate sections.

Organize tables and figures to facilitate comparisons, grouping related data in as few tables and figures as feasible. As far as possible, make the tables and figures clear without reference to the text.

Begin sections and paragraphs with topic sentences containing generalizations that lead readily to the particulars. Giving a conclusion first and then supporting it not only improves readability but also facilitates assessment by other scientists. Failure to give the most newsworthy generalizations first is one of the most prominent shortcomings in presentation of manuscripts.

See that everything in each section is relevant to the heading, and everything in each paragraph to the topic (opening) sentence.

Before writing any paragraphs, try writing the topic sentences for all of them and arranging these in appropriate order.

Title

Limit the title to what is documented in the manuscript. It is the key to the article and should clearly and concisely reveal what appears in the paper itself. The title serves two functions: (1) it allows the reader to judge whether or not the article is of potential interest and (2) it should provide enough information to permit the reader to judge the scope and potential importance of the article. Words in the title should convey a maximum amount of information and identify the nature of the research, organism used, and where appropriate, the technical approach (e.g. X ray, chromatography, mathematical analysis). Titles should not begin with a numeral or introductory prepositions such as "On" or "Towards" or expressions such as "A contribution to . . ." or "Investigations on . . ." Good titles greatly assist scientists and librarians in using scientific literature and aid indexers preparing titles for keyword indexes.

Abstract

The abstract should succinctly mirror the content and emphasis of the paper in about 175 words. Like the title, the abstract enables readers to determine the paper's content and decide whether or not they need to read the entire article. Begin the abstract with the main conclusion from the study, and support it with the relevant findings. Limit details of methods to those needed in understanding what was done, and work them into statements of findings. Avoid using phrases such as ". . . is discussed" or ". . . was found"; be specific. As the abstract

is often divorced from the main body of the paper by abstracting and indexing services and is the only part of a paper some readers ever see, it is important that it accurately reflect the paper's contents and be completely self-contained (i.e. any *essential* references) in a retrievable form (e.g. R. B. Deriso. 1980. Can. J. Fish. Aquat. Sci. 37: 268–282).

Introduction

Limit the introduction largely to the scope, purpose, and rationale of the study. Restrict the literature review and other background information to that needed in defining the problem or setting the work in perspective. Try beginning with the purpose or scope of the work, defining the problem next, and adding guideposts to orient the reader. An introduction generally need not exceed 375–500 words.

Materials and Methods

Materials and Methods provides the framework for getting answers to the questions posed in the purpose of the work.

Limit the information on materials and methods to what is needed in judging whether the findings are valid. To facilitate assessment, give all the information in one section when possible. Refer to the literature concerning descriptions of equipment or techniques already published, detailing only adaptations. Often, it helps to begin statements on procedures with a phrase indicating the purpose, such as "To determine . . . we . . ." If the section is long, consider using subheadings corresponding to headings for the findings.

Results

Limit the results to answers to the questions posed in the purpose of the work, and digest them as comprehensively as possible. Give the findings as nearly as possible in the terms in which the observations or measurements were made and so avoid confusion between facts and inferences. State noteworthy findings to be noted in each table and figure, and avoid restating in the text what is clear from the titles. Material supplementary to the text can be archived in the report literature or a recognized data depository and referenced in the text.

Discussion

Limit the discussion to giving the main contributions of the study and interpreting particular findings, comparing them with those of other workers. Emphasis should be maintained on synthesis and interpretation and exposition of broadly applicable generalizations and principles. If these are exceptions or unsettled points, note them and show how the findings agree or contrast with previously published work. Limit speculation to what can be supported with reasonable evidence. End the discussion with a short summary of the significance of the work and conclusions drawn. If the discussion is brief and straightforward, it can be combined with the results section.

Acknowledgments

We strongly urge authors to limit acknowledgments to those who contributed substantially to scientific and technical aspects of the paper, gave financial support, or improved the quality of the presentation. Avoid acknowledging those whose contribution was clerical only.

References

References should be selected judiciously and be largely restricted to significant, published literature. References to unpublished data, manuscripts in preparation or submitted to other journals, progress reports, and unpublished papers given at annual meetings may not be cited in the reference list but may be noted in the text as unpublished data or personal communications (include mailing addresses). If consultants' reports or other documents of limited circulation must be cited, they should carry with them an availability statement explaining where the document can be obtained. Citations of literature in the text should be carefully checked against those in the reference list and vice versa to ensure exact correspondence. Nearly every manuscript submitted to the *Journal* contains errors in the references.

Tables

Tables are used to present repetitive data and should be as economical of space as possible.

Design tables to fit a 1-, 1½-, or 2-column width of the *Journal*.

Type each on a separate page and number with arabic numerals.

Use horizontal lines above and below the headings and below the columns, and seldom elsewhere. Never use vertical lines; leave extra spaces instead.

Table captions should be succinct and identify the purpose of the table sufficiently well to allow the table to stand on its own.

Indicate table footnotes by superscript small letters and type them below the table.

Place the tables after the list of references.

Where each table is first referred to, type in the margin "Table — near here."

Note that *text tables* are not numbered, are typed with the text, and seldom need horizontal lines.

Figures

Provide photographic reproductions, laser printouts, or the original artwork (no larger than 8.5 × 11 in.) of each illustration. If submitting photographs, type the author's name and figure name on a strip of paper and fasten to the back of the print. Do not write on the illustration itself. Provide two photocopies of line illustrations and two sets of any photographs for review purposes.

Design figures to fit a 1-, 1½-, or 2-column width of the *Journal* and ensure that all lettering, numerals, and symbols are legible, neat, and at least 1.5 mm high when reduced. Figures should not be mounted, stapled, or clipped. When possible, figures should be grouped and be done with the same or similar type of lettering.

Type legends in succession on a separate page. Add bar scales to maps, photomicrographs, equipment diagrams, etc.

Where each figure is first referred to, type "Fig. — near here."

Computer-generated figures produced on laser printers are acceptable; figures produced on dot matrix printers are not acceptable. If sheets of printed characters are used, be sure they adhere completely.

Draft rather than type axis labels for graphs.

Color plates are very expensive to produce and should only be used where the color being presented is essential to the

understanding of the text. Extra costs for the reproduction of color plates are usually charged to the author. Submit only the most outstanding photographs for color reproduction. We prefer to use color photographs, but will accept drawings, color negatives, slides, or film positives.

Mathematical Expressions, Equations, and Formulae

Leave three spaces above and below lines that have much material in superscripts, and above and below equations and formulae. In the various expressions, place each component in its correct position.

Show all symbols clearly. In the margin, spell out Greek letters the first time they occur, and distinguish between the small letter *l* and the number *one*, and between the capital *O* and the numeral *zero*. Make other explanatory notes similarly.

Appendix

Abbreviations

Abbreviate terms denoting units of weight and measurement in the text only when they are preceded by numerals.

becquerel	Bq
calorie	cal
centimetre	cm
centimetre, square	cm^2
centimetre, cubic	cm^3
centimetres per gram per second	$cm \cdot g^{-1} \cdot s^{-1}$
coulomb	C
day	d
decimetre	dm
degree Celsius	°C
degrees of freedom	df
gram	g
hectare	ha
hertz	Hz
hour	h
joule	J
kilometre	km
litre	L
lumen	lm
lux	lx
metre	m
metre, square	m^2
metre, cubic	m^3
micrometre	µm
milligram	mg
millilitre	mL
millimetre	mm
millimetre, square	mm^2
minute	min
molar mass	M
mole	mol
moles per litre	mol/L, M
month	mo
Pascal	Pa
second	s
standard deviation	SD
standard error	SE
tonne	t
volt	V
volume	vol
watt	W
week	wk
year	yr

Dates

Dates may be written in the sequence day-month-year without internal punctuation (On 9 October 1983 the . . .), or the alternative sequence month-day-year may be employed with the year set off by commas before and after it (On October 9, 1983, the . . .).

Reference Citations in Text

Name-and-year system

The *Journal* uses the name-and-year system of citation; that is, the surname of the author(s) and the year of publication are inserted in the text at an appropriate point: "Brown (1983) compared . . ." or ". . . were compared (Brown 1983)." If the reference has more than two authors, include only the surname of the first author followed by "et al." (not italicized): "Brown et al. (1983) compared . . ." or ". . . were compared (Brown et al. 1983)."

Personal communications

Personal communications are not listed in the reference list. Using parentheses in the text, state the name and mailing address of the communicator followed by "pers. comm."

Unpublished data

If the unpublished book or article has been accepted for publication, list it in the reference list section followed by the notation "(In press)." Only those manuscripts that are in galley or page proof or for which there is an acceptance letter can be considered in press. If the article is submitted but not yet accepted, state the name and address of the author of the unpublished material followed by the notation "unpubl. data" in the text and do not include it in the reference list.

Reference Lists

The following bibliographic citations illustrate the punctuation, style, and abbreviations (according to *Serial Sources for the BIOSIS Data Base*) for references.

Journal article

PETERMAN, R. M. 1982. Model of salmon age structure and its use in preseason forecasting and studies of marine survival. Can. J. Fish. Aquat. Sci. 39: 1444–1452.

Entire issue of journal

GORDON, D. C. JR., AND A. S. HOURSTON [ED.] 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. 40(Suppl. 1): 1–365.

Book in a series

SCOTT, W. B., AND E. J. CROSSMAN. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 966 p.

Book not in a series

LEBLOND, P. H., AND L. A. MYSAK. 1978. Waves in the ocean. Elsevier, New York, NY. 602 p.

Part of book

HEALEY, M. C. 1980. The ecology of juvenile salmon in Georgia Strait, British Columbia, p. 203–229. *In* W. J. Neil and D. C. Himsworth [ed.] Salmonid ecosystems of the North Pacific. Oregon State University Press, Corvallis, OR.

Corporate author

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1975. Standard methods for the examination of water and wastewater. 14th ed. Washington, DC. 1193 p.

Theses

KUTTY, M. N. 1966. Some studies on the respiratory quotient in goldfish and rainbow trout. Ph.D. thesis, University of Toronto, Toronto, Ont. 102 p. Natl. Libr. Can., Can. Theses Microfilm No. 646.

Reports

SMITH, J. E. 1981. Catch and effort statistics of the Canadian groundfish fishery on the Pacific coast in 1980. Can. Tech. Rep. Fish. Aquat. Sci. 1032: 90 p.

Translation

KOIKE, A., AND M. OGURA. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. 64: 1–11. (Transl. from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983)

Time

A colon should be used as the separator between hour and minute and between minute and second. The symbols "h," "min," and "s" are not used, since they are the symbols for hour, minute, and second in the sense of duration or the length of time. Thus, "12 h 30 min" expresses a measured time of twelve hours and thirty minutes duration whereas 12:30 refers to the time of day.

Word List

The spelling of the following words is frequently inconsistent in submitted manuscripts. We prefer that authors adhere to the *Journal's* house style for these commonly used terms:

age-class (n.)
aquaculture (n.)
Arctic char (n.)
brackish water (n.)
brackish-water (adj.)
chi-square (n., adj.)
cold water (n.)
cold-water (adj.)
deep sea (n.)
deep-sea (adj.)
deep water (n.)
deepwater (adj.)
freshwater (n., adj.)
fresh water (n.)
groundwater (n., adj.)
hard water (n.)
hardwater (adj.)
headwater (n., adj.)
lake water (n., adj.)
meltwater (n., adj.)
open water (n.)
open-water (adj.)
percent (n.)
salt water (n.)
saltwater (adj.)
sea-run (adj.)
seawater (n., adj.)
shallow water (n.)
shallow-water (adj.)
short term (n.)
size-class (n.)
snowmelt (n.)
soft water (n.)
softwater (adj.)
tidewater (n., adj.)
t-test (n., adj.)
warm water (n.)
warmwater (adj.)
year-class (n.)
young-of-the-year (n., adj.)

Guide des auteurs

Introduction

Les manuscrits comportant une information nouvelle dans le domaine des sciences halieutiques et aquatiques sont les bienvenus. Ils peuvent se rapporter aux cellules, aux organismes, aux populations, aux écosystèmes ou aux processus qui influencent les systèmes de production aquatiques, et couvrir de nombreuses disciplines, y compris biologie et écologie des organismes marins et dulçaquicoles, physiologie, toxicologie, génétique, limnologie, océanographie, économique, maladies et gestion.

Les critères de sélection des manuscrits dépendent de l'étendue et de l'importance des connaissances ou idées nouvelles qu'ils contiennent. On donnera la préférence à ceux dont les résultats expérimentaux font ressortir les mécanismes sous-jacents aux phénomènes observés plutôt qu'une simple description ou énoncé de fait. Ils doivent aboutir à des conclusions ou synthèses précises qui, de diverses façons, peuvent accroître, modifier, remettre en question ou réorienter le bagage actuel des connaissances et perceptions dans une discipline donnée des sciences aquatiques. L'originalité doit dépasser le caractère particulier (une année, un endroit, un taxon ou un composé chimique donné) et tenir à une épuration ou à une reformulation des connaissances actuelles en faisant plus que corroborer des faits.

Les manuscrits soumis devraient être aussi complets que possible; des fragments de recherche sont généralement inacceptables. S'il est impossible de produire un seul article, on devra soumettre ensemble des articles étroitement liés entre eux, avec références croisées. L'article ne doit pas avoir été soumis ou accepté pour publication ailleurs.

Le *Journal* considère les types de contributions suivants :

Articles : études d'une portée générale, constituant des contributions inédites à la science.

Critiques : appréciations critiques détaillées de vastes champs de recherche, particulièrement dans les domaines ayant connu de rapides progrès au cours des dernières années.

Perspectives : essais portant sur une opinion ou une hypothèse relative aux sciences aquatiques qui intéressent professionnels et profanes. Il est préférable de donner des références et d'écrire, si possible, dans une style simple.

Commentaires : opinions sur des sujets récemment exposés dans le *Journal*, et, de préférence, ne dépassant pas 500 mots.

Soumission des manuscrits

Les manuscrits doivent être soumis au rédacteur en triplicata et accompagnés d'une lettre qui fait était 1) des principaux points et de l'importance du travail, 2) de la participation de tous les auteurs au manuscrit, de leur approbation de ce dernier et de l'assurance que le manuscrit en question n'est pas en cours d'examen au fins de publications dans une autre revue, 3) de suggestions quant aux arbitres éventuels et 4) d'une mention d'autres manuscrits contenant une information identique ou qui se rapproche de celle contenue dans le manuscrit soumis. L'auteur devra, de préférence, conserver les originaux du texte dactylographié et des figures (sauf les simili) jusqu'à ce qu'on les lui demande. Afin de les protéger lors de l'expédition, les manuscrits et les illustrations doivent être solidement emballés.

Un manuscrit est normalement soumis à deux arbitres pour évaluation, avant l'évaluation finale par un rédacteur. Cependant, les manuscrits qui ne répondent pas à la politique éditoriale ou à la portée du *Journal* sont retournés à l'auteur sans avoir été soumis à l'arbitrage.

Dispositions générales

Le *Council of Biology Editors Style Manual* (5e éd., CBE, 9650 Rockville Pike, Bethesda, MD 20814) sert de guide quant à la forme de la présentation. Le *Webster's Third International Dictionary* ou l'*Oxford English Dictionary*, et le *Grand Larousse encyclopédique* font autorité pour l'orthographe des mots. L'uniformité de cette dernière est la responsabilité des auteurs. Les titres de périodiques sont abrégés suivant les *Serial Sources for the BIOSIS Data Base* (BioSciences Information Service, Philadelphia, PA 19103). C'est la responsabilité des auteurs de s'assurer que leurs références sont complètes et exactes. Pour les noms de poissons et leur orthographe, le *Journal* suit les recommandations contenues dans *A List of Common and Scientific Names of Fishes from the United States and Canada* (5e éd. 1991. Spec. Publ. No. 20, American Fisheries Society). Pour la nomenclature génétique pour les locus de codage des protéines, le *Journal* suit les recommandations contenues dans Shaklee *et al.* (1990. Trans. Am. Fish. Soc. 119 : 2–15). Toutes les mesures doivent être conformes au système métrique et, dans la plupart des cas, être exprimées en unités SI (sauf exceptions mentionnées dans un éditorial du *Journal*, vol. 40, no 12), et se conformer au *Guide canadien de familiarisation au système métrique* CAN3-Z234.1-79 (l'Association canadienne de normalisation, 178, boul. Rexdale, Rexdale (Ont.) M9W 1R3).

On devra éviter les titres en séries. Un résumé doit accompagner chaque texte et exposer brièvement, en 175 mots ou moins, ce qui a été fait, ce qui a été découvert et les conclusions auxquelles on est arrivé.

L'auteur doit revoir les épreuves qu'on lui remet et en corriger les erreurs typographiques. *Il doit éviter de modifier le contenu du texte.*

Préparation des manuscrits

Directives générales

La marche des manuscrits à travers les diverses étapes de l'arbitrage et de la révision sera grandement facilitée si les articles sont préparés dans le style et le format du *Journal*. En cas de doute sur des points particuliers, veuillez consulter une livraison récente du *Journal*. On trouvera ci-dessous des directives générales.

Dactylographiez le manuscrit sur papier blanc, mesurant de préférence 8,5 sur 11 pouces, sur un côté de la page. Laissez toujours une marge d'au moins 1 pouce.

Sur la première page, dactylographiez le titre, les noms et affiliations des auteurs et les renvois qui s'y rapportent. Numérotez toutes les pages, à commencer par la page-titre, de même que celles contenant les tableaux et les légendes des illustrations.

Tout le manuscrit, y compris tableaux, légendes des tableaux et des figures, renvois et liste de références, doit être dactylographié à double interligne. Ne soulignez que les mots qui devront être imprimés en italique.

En aucun droit du manuscrit n'écrivez entièrement en majuscules.

Pour les références citées dans le texte, utilisez le système nom et année.

Liste de contrôle pour manuscrits

Une attention particulière aux questions suivantes facilitera l'évaluation des manuscrits par les arbitres et les rédacteurs.

Les découvertes, interprétations et conclusions sont-elles bien documentées et pertinentes aux buts de l'étude ?

Les tableaux et figures sont-ils tous nécessaires et agencés pour faciliter les comparaisons ? Y a-t-il contradiction entre les tableaux ou les figures et le texte, ou à l'intérieur du texte ? Est-ce que certaines données devraient être présentées séparément dans un rapport manuscrit ou dans un dépôt de données ?

Si vous faites une analyse statistique, la statistique cède-t-elle le pas à la recherche ? Dans des énoncés de probabilité, seuls les tests statistiques sont-ils cités, et les tableaux statistiques inutiles sont-ils exclus ?

Le texte y gagnerait-il en clarté s'il plus condensé, en tout ou en partie ? Contient-il des exposés sommaires au début de chaque section ou paragraphe, et les détails de ces sections et paragraphes sont-ils pertinents au sujet traité ? La marche du manuscrit est-elle conforme à la logique énoncée dans l'introduction ?

Le *titre* est-il représentatif du contenu du rapport ? Le *résumé donne-t-il l'essentiel des connaissances nouvelles ? L'introduction* se limite-t-elle à une esquisse de la portée, du but et des raisons de l'étude ? La revue de la littérature se limite-t-elle à la définition du problème ? La section *matériel et méthodes* contient-elle le minimum de détail qui permettront aux autres scientifiques de saisir les grandes lignes de l'étude et de juger de la valeur des données ? Les généralisations sur les *résultats* sont-elles adéquates ? Permettent-elles de distinguer entre constations et déductions ? La *discussion* est-elle limitée à l'interprétation et à l'évaluation des découvertes ?

Sont énumérés ci-dessous des façons d'éviter les problèmes techniques les plus communs dans la soumission des manuscrits. En accordant une attention particulière à ces points lors de la préparation de leurs manuscrits, les auteurs peuvent accélérer le traitement des articles.

1) Insérez la bande bibliographique avant le résumé (auteurs, date, titre, Can. J. Fish. Aquat. Sci.)

2) Limitez le résumé à un paragraphe d'environ 175 mots.

3) Dactylographiez à double interligne tous les éléments du manuscrit, y compris les références et les légendes des tableaux et des figures.

4) Évitez partout l'emploi exclusif de majuscules, y compris dans les en-têtes et les légendes des tableaux et des figures.

5) Ne soulignez que les noms latins d'organismes et certaines notations statistiques et mathématiques, qui devront être imprimés en italique.

6) Dans les figures, les tableaux, et le teste, exprimez les unités de mesure par leurs symboles SI propres. Pour les nombres inférieurs à l'unité, placez un zéro avant la virgule décimale.

7) Évitez les formes ambiguës, telles que g $C/m^2/d$; utilisez plutôt g $C \cdot m^{-2} \cdot d^{-1}$.

8) Identifiez le test qui a servi à évaluer la signification statistique, donnant également la valeur de probabilité. S'il s'agit de tests communs, il n'est pas nécessaire d'en donner la référence.

9) Dans le texte, généralisez à partir des tableaux et des figures ; évitez une répétition de tous les détails. Assurez-vous que tous les tableaux et figures sont indépendants et que, dans le texte, on y réfère dans l'ordre numérique. Les légendes doivent indiquer le but du tableau ou de la figure.

10) Préparez la section des remerciements au stade du manuscrit et non quand vous parviennent les épreuves.

11) En référant à des communications personnelles, donnez les initiales et l'adresse postale de la personne en cause.

12) Éliminez toutes références inutiles qui ne s'appliquent pas directement au problème.

13) Vérifiez soigneusement les références en regard des citations dans le texte et vice versa, afin de vous assurer qu'il y a correspondance. Dans le cas de matériel difficilement accessible, indiquez l'endroit où le trouver, p. ex., disponible auprès du Département d'économique, Université Simon Fraser, Burnaby (C.-B.) V5A 1S6.

14) Éliminez les virgules entre le nom et la date dans les citations telles que (Smith 1900) et soulignez *et al.*

15) Dans les en-têtes de tableaux et les axes des figures, remplacez 10^n par les préfixes SI appropriés ou utilisez des mots en clair (p. ex. milliers).

16) Aux fins de la révision, les photocopies des figures, sauf les similis, sont appropriées. Sur demande, lorsqu'il s'agira de publier l'article, envoyez des photographies ou des imprimés laser des figures, ou encore les originaux, s'ils ne dépassent pas 8,5 sur 11 pouces. Utilisez autant que possible un lettrage uniforme.

Parties du manuscrit

Organisation

Organisez le manuscrit par rapport au but ou à la portée de l'étude, tels qu'énoncés dans l'introduction. Il est particulièrement important que le titre et les sous-titres soient en harmonie avec les objectifs.

Avant de commencer la rédaction du manuscrit, dressez une liste provisoire des sous-titres ou en-têtes, avec le moins de divisions possible. Retouchez-les jusqu'à ce qu'ils semblent assurer, pour le lecteur, une marche logique ; ordinairement, l'ordre chronologique ne sera pas satisfaisant. Il sera plus facile d'évaluer les résultats si vous décrivez méthodes, résultats et discussion dans des sections séparées.

Organisez les tableaux et les figures de façon à faciliter les comparaisons, groupant les données apparentées en aussi peu de tableaux et de figures que possible. Assurez-vous que ces derniers sont aussi clairs que possible sans référence au texte.

Commencez les sections et les paragraphes par des énoncés généraux qui aboutissent naturellement à des énoncés particuliers. Si vous donnez d'abord une conclusion que vous appuyez ensuite, non seulement votre travail sera plus facile à lire, mais aussi les autres scientifiques pourront plus aisément en évaluer les résultats. Dans la rédaction des manuscrits, le défaut le plus commun est d'omettre de donner, au début, les généralités les plus dignes de mention.

Voyez à ce que le matériel de chaque section soit pertinent à l'en-tête et que le matériel de chaque paragraphe se rapporte à la première phrase introduisant le sujet.

Avant de rédiger les paragraphes, composez la phrase-thème de chaque paragraphe et disposez ces phrases dans l'ordre approprié.

Titre

Limitez le titre à ce que le travail veut démontrer. C'est la clé de l'article, et il devrait montrer clairement et de façon concise ce qui est contenu dans le travail lui-même. Le titre a deux fonctions : 1) il permet au lecteur de juger si, oui ou non, l'article peut avoir un intérêt et 2) il devrait fournir suffisamment d'information pour permettre au lecteur de juger de la portée et de l'importance possible de l'article. Les mots du titre devraient fournir un maximum d'information et identifier la nature de la recherche, de l'organisme utilisé et, au besoin, la méthode d'approche technique (p. ex. rayons X, chromatographie, analyse mathématique). Les titres ne devraient pas commencer par un chiffre ou par des prépositions d'introduction telles que «Sur» ou «Vers», ou des expressions telles que «Contribution à . . .» ou «Recherche sur . . .». De bons titres facilitent le classement des publications et leur utilisation par les scientifiques et les bibliothécaires.

Résumé

Le résumé doit refléter succintement le contenu et les points principaux de l'article en 175 mots environ. Comme le titre, le résumé permet aux lecteurs de prendre connaissance du contenu de l'article et de décider si, oui ou non, il leur faut lire ce dernier en entier. Indiquez au début du résumé la principale conclusion de l'étude et appuyez-la par des découvertes pertinentes. Ne donnez que les détails des méthodes nécessaires à la compréhension de ce qui a été fait et insérez-les dans l'énoncé des découvertes. Évitez l'emploi de phrases telles que «. . . on y analyse» ou «. . . a été trouvé» ; soyez spécifique. Comme le résumé est souvent séparé du corps principal de l'article par les services d'analyse et de classement, et que c'est la seule partie d'un travail que certains lecteurs auront l'occasion de voir, il est important que le résumé reflète précisément le contenu du travail et qu'il soit complet en lui-même (c.-à.-d. seule les references *essentielles* sont indiquées, sous forme retraçable, p. ex. R. B. Deriso. 1980. J. can. sci. halieut. aquat. 37 : 268–282).

Introduction

Limitez l'introduction à la portée, au but et à la raison de l'étude. Ne mentionnez que les travaux publiés et autres informations nécessaires à la définition du problème et à la perspective du travail. Commencez avec le but ou la portée du travail, définissez ensuite le problème et ajoutez ici et là des jalons qui aideront à orienter le lecteur. En général, une introduction n'a pas besoin de dépasser 375-500 mots.

Matériel et méthodes

Cette section renferme ce dont on a besoin pour trouver réponse aux questions soulevées dans les objectifs du travail.

Limitez les renseignements à ce qui est nécessaire pour juger de la validité des découvertes. Cette évaluation sera facilitée si vous présentez, autant que possible, toute l'information dans une section. S'il s'agit d'outillage ou de techniques déjà publiés, référez à ces publications et ne décrivez en détail que les adaptations. Il y a souvent avantage à commencer un énoncé des méthodes par une phrase qui en indique le but, comme : «Afin de déterminer . . . nous . . .». Si la section est longue, songez à utiliser des sous-titres correspondant à ceux que vous utilisez dans la section des résultats.

Résultats

Limitez les résultats aux réponses apportées aux questions en rapport avec le but de l'étude ; que ces réponses soient aussi complètes que possibles, tout en étant concises. Exprimez ce que vous avez trouvé, autant que possible dans les mêmes termes que ceux dans lesquels les observations ou les mesures ont été faites, évitant ainsi de confondre faits et spéculations. En dégageant les généralités des tableaux et des figures, référez-y et évitez de répéter dans le texte ce que les légendres devraient exprimer clairement. Le matériel complémentaire au texte peut être classé dans les séries des rapports ou dans un dépôt de données reconnu, et on y référera dans le texte.

Discussion

Limitez la discussion à l'énoncé de la principale contribution de l'étude et à l'interprétation de découvertes particulières, les comparant avec celles d'autres auteurs. On continuera à mettre l'accent sur la synthèse et l'interprétation, ainsi que sur l'exposition de généralisations et de principes largement applicables. S'il s'agit d'exceptions ou de points douteux, notez-les et faites ressortir la manière dont vos résultats corroborent ou contredisent les travaux déjà publiés. Limitez la spéculation à ce que vous pouvez appuyer sur des preuves raisonnables. Terminez cette section par un court résumé de l'importance du travail et des conclusions auxquelles il a conduit. Si la discussion est brève et précise, elle peut être combinée avec les résultats en une seule et même section.

Remerciements

Nous recommandons fortement aux auteurs de limiter les remerciements aux personnes qui ont contribué d'une manière substantielle aux aspects scientifiques et techniques de l'article, fourni une aide financière ou amélioré la qualité de la présentation. Évitez de mentionner les personnes qui n'ont fourni qu'un travail de soutien.

Références

On choisira les références avec soin, les limitant aux publications importantes. On ne peut citer dans cette section les références à des données inédites, manuscrits en préparation ou soumis à d'autres périodiques, états des travaux et communications inédites présentées à des réunions annuelles. On peut toutefois les mentionner dans le texte à titre de données inédites ou de communications personnelles (incluant les adresses). Si l'on doit citer des rapports d'experts-conseils ou d'autres documents dont la distribution est limitée, on devra préciser l'endroit où l'on peut se les procurer. On doit s'assurer que les références mentionnées dans le texte correspondent à celles qui sont contenues dans la liste de références et vice versa. Presque tous les manuscrits soumis au *Journal* contiennent des erreurs dans cette section.

Tableaux

Les tableaux sont utilisés pour présenter des données qui se répètent et devraient occuper le moins d'espace possible.

Agencez les tableaux de façon qu'ils s'adaptent soit à 1, 1½- ou 2 colonnes du *Journal*.

Dactylographiez chaque tableau sur une page séparée et numérotez-les en chiffres arabes.

Tracez des lignes horizontales de part et d'autre des en-têtes et au-dessous des colonnes, mais rarement ailleurs. N'utilisez jamais de lignes verticales. Laissez plutôt des espaces supplémentaires.

Chaque tableau doit avoir une légende instructive, complète et indépendante.

Les renvois des tableaux doivent être indiqués par des petites lettres suscrites dactylographiées au bas du tableau.

Placez les tableaux après la liste des références.

Quand on réfère à un tableau pour la première fois, on indique dans la marge du manuscrit «Tableau — près d'ici».

Notez que les *tableaux dans le texte* ne sont pas numérotés et sont dactylographiés dans le corps du texte. Ils ont rarement besoin de lignes horizontales.

Figures

Fournissez des photographies, des imprimés laser ou les dessins originaux (pourvu qu'ils ne dépassent pas 8,5 sur 11 pouces) de chaque illustration. Dactylographiez le nom de l'auteur et le numéro de la figure sur une lisière de papier que vous collerez au dos. N'écrivez pas sur l'illustration même. Transmettez deux photocopies des dessins au trait et deux séries de photos qui serviront au moment de la révision.

Les dessins devront occuper une largeur de 1, 1½- ou 2 colonnes du *Journal*. Assurez-vous que tous les chiffres, lettres et symboles sont lisibles, clairs et mesurent au moins 1,5 mm de hauteur une fois réduits. Les figures ne devraient pas être montées, brochées ou fixées à l'aide de trombones. Autant que possible, elle devraient être groupées et préparées avec le même type de lettrage.

Dactylographiez les légendes, dans l'ordre, sur des feuilles séparées. Dans le cas de cartes, photomicrographies, diagrammes d'instruments, etc., ajoutez une échelle.

À l'endroit où chaque figure est mentionnée pour la première fois, inscrivez en marge «Fig. — près d'ici».

Les illustrations produites par imprimantes laser sont acceptable; les illustrations produites par imprimantes par points ne sont pas acceptable. Si vous utilisez du lettrage instantané, veillez à ce qu'il adhère parfaitement.

Il vaut mieux dessiner que dactylographier les inscriptions relatives aux axes.

La production de planches en couleurs est très dispendieuse et on ne devrait utiliser celles-ci que lorsque les couleurs sont essentielles à la compréhension du texte. Les coûts supplémentaires de reproduction de planches en couleurs sont ordinairement débités à l'auteur. Pour les reproductions en couleurs, ne soumettez que des photos d'excellerte qualité. Nous préférons utiliser des photographies en couleur, mais acceptons dessins, négatifs, diapositives ou positifs de films en couleurs.

Expressions, équations et formules mathématiques

Laissez trois espaces au-dessus et au-dessous des lignes portant des chiffres ou des lettres suscrits et souscrits, ou encore au-dessus et au-dessous des équations et des formules. Placez chaque composante des différentes expressions dans la position qui lui est propre.

Indiquez clairement tous les symboles. Épelez en marge les lettres grecques, la première fois qu'elles apparaissent. Faites une distinction entre la lettre *l* et le chiffre *un*, et entre la majuscule *O* et le chiffre *zéro*. Insérez de la même façon toute autre note explicative.

Appendice

Abréviations

N'abrégez les termes désignant les unités de poids et mesures dans le texte que lorsqu'ils sont précédés d'un nombre.

an	an
année	a
becquerel	Bq
calorie	cal
centimètre	cm
centimètre carré	cm^2
centimètre cube	cm^3
centimètres par gamme par seconde	$cm \cdot g^{-1} \cdot s^{-1}$
coulomb	C
décimètre	dm
degré Celsius	°C
degrés de liberté	dl
écart type	ET
électronvolt	eV
erreur type	ErT
farad	F
gramme	g
hectare	ha
hertz	Hz
heure	h
joule	J
jour	d
kilomètre	km
litre	L
lumen	lm
lux	lx
mètre	m
mètre carré	m^2
mètre cube	m^3
micromètre	µm
milligramme	mg
millilitre	mL
millimètre	mm
millimètre carré	mm^2
minute	min
mois	mo
masse molaire	M
mole	mol
moles par litre	mol/L, M
pascal	Pa
seconde	s
semaine	sem
tonne	t
volt	V
volume	vol
watt	W

Dates

On écrira les dates dans l'ordre jour-mois-année, p.ex. 9 octobre 1983, sans virgule entre le mois et l'année.

Citations de références dans le texte

Système de nom et année

Le *Journal* utilise le système de nom et année, c.-à-d. que le nom de l'auteur ou des auteurs et l'année de publication sont

insérés dans le texte à un endroit approprié : «Brown (1983) compare . . .» ou «. . . on a comparé (Brown 1983)». Si la référence comporte plus de deux auteurs, inscrivez le nom du premier auteur seulement, suivi de «*et al.*» : «Brown *et al.* (1983) comparent . . .» ou «. . . on a comparé (Brown *et al.* 1983)».

Communications personnelles

Les communications personnelles ne doivent pas être incluses dans la liste des références. Utilisez plutôt des parenthèses dans le texte, entre lesquelles vous indiquerez le nom et l'adresse de la personne en question, suivis de «comm. pers.».

Données inédites

Si le livre ou l'article inédits ont été acceptés pour publication, insérez-le dans la liste des références, avec la mention «(Sous presse)». On ne peut considérer comme étant sous presse que les manuscrits au stade d'épreuves ou ceux couverts par lettre d'acceptation. Si l'article a été soumis mais n'a pas encore été accepté, ne l'incluez pas dans la liste de références, mais indiquez le nom et l'adresse de l'auteur du matériel inédit, dans le texte lui-même, avec la mention «données inédites».

Liste de références

La liste qui suit donne des exemples de ponctuation, de style et d'abréviations utilisés dans les références bibliographiques (d'après les *Serial Sources for the BIOSIS Data Base*).

Article du journal

ARCHAMBAULT, D. ET E. BOURGET. 1983. Importance du régime de dénudation sur la structure et la succession des communautés intertidales de substrat rocheux en milieux subarctique. Can. J. Fish. Aquat. Sci. 40 : 1278–1292.

Numéro complet du journal

GORDON, D. C. JR., AND A. S. HOURSTON [ÉD.] 1983. Proceedings of the Symposium on the Dynamics of Turbid Coastal Environments. Can. J. Fish. Aquat. Sci. 40(Suppl. 1) : 1–365.

Livre dans une série

HILTON, J. W. ET S. J. SLINGER. 1981. Nutrition et alimentation de la truite arc-en-ciel. Publ. spéc. can. sci. halieut. aquat. 55 : 17 p.

Livre ne faisant pas partie d'une série

FORRESTER, W. D. 1983. Manuel canadien des marées. Ministère des Pêches et des Océans. Service hydrographique du Canada, Ottawa (Ont.). 148 p.

Partie d'un livre

AUBERT, M., J. AUBERT, S. DANIEL ET N. DESIROTTE. 1982. État actuel de la pollution bactérienne au large des côtes françaises, p. 36–40. *Dans* M. Ruivo [éd.] La pollution des mers et les ressources biologiques. Fishing News (Books) Ltd., Londres.

Corporation-auteur

AMERICAN PUBLIC HEALTH ASSOCIATION, AMERICAN WATER WORKS ASSOCIATION, AND WATER POLLUTION CONTROL FEDERATION. 1975. Standard methods for the examination of water and wastewater. 14th ed. Washington, DC. 1193 p.

Thèse

DELISLE, C. 1969. Écologie, croissance et comportement de l'éperlan du Lac Heney, Comté de Gatineau ainsi que la répartition en eau douce du Québec. Thèse doctorale, Université d'Ottawa, Ottawa (Ont.). 161 p.

Rapports

DUTIL, D. J., M. FORTIN ET Y. VIGNEAULT. 1982. L'importance des zones littorales pour les ressources halieutiques. Mémoire remis au Conseil consultatif de l'environnement du Québec. Rapp. manuscr. can. halieut. aquat. 1653 : 29 p.

Traduction

KOIKE, A., AND M. OGURA. 1977. Selectivity of meshes and entrances of shrimp traps and crab traps. J. Tokyo Univ. Fish. 64 : 1–11. (Transl. from Japanese by Can. Transl. Fish. Aquat. Sci. 4950, 1983)

Temps

On devra séparer par deux points (:) heures et minutes, et minutes et secondes. Les symboles «h,» «min» et «s» ne seront pas utilisés car ils représentent heure, minute et seconde dans le sens de durée ou intervalle de temps de douze heures et trente minutes, alors que 12 : 30 se rapporte à l'heure du jour.

Liste de mots

Cette section, dans la version anglaise du Guide, contient une série de mots dont l'orthographe pose certains problèmes d'uniformité, comme, par exemple, «cold water» (n.) «cold-water» (adj.); «hard water» (n.), «hardwater» (adj.), etc. L'auteur préparant un texte en anglais est prié de consulter la version anglaise du Guide.

1993

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CJFSDX50(1) 1–222 (1993)
ISSN 0706-652X

Canada

Typeset and printed in Canada by/
Compose et imprime au Canada par
Lowe-Martin Group: Runge Division

0706-652X(199301)50:1;1-W